g W, Dasgupta R, Rueckert R.
. Evidence that the packaging
1 for nodaviral RNA2 is a bulged
-loop. *Proc. Natl. Acad. Sci. USA*
1146–50
nern D. 1977. The nucleotide
ence at the origin for assembly on
cco Mosaic virus RNA. *Cell* 11:
82
nern D. 1988. Evolution of RNA
es. In *RNA Genetics*, ed. D Es-
i, JJ Holland, P Ahlquist, 2:211–
Boca Raton: CRC Press

THE FUNCTION OF HEAT-SHOCK PROTEINS IN STRESS TOLERANCE: DEGRADATION AND REACTIVATION OF DAMAGED PROTEINS

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                tolerance

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INTRODUCTION

Organisms respond to sudden increases in temperature by synthesizing a small set of proteins called the heat-shock proteins (hsps) (See Figure 1). This heat-shock response has been highly conserved throughout evolution, not only as a physiological phenomenon, but also at the level of the individual proteins. Hsps comprise some of the most highly conserved protein families known. The level of amino-acid identity between all prokaryotic and eukaryotic hsp70 proteins, for example, approaches 50% (95). Hsp families often include constitutive as well as heat-inducible
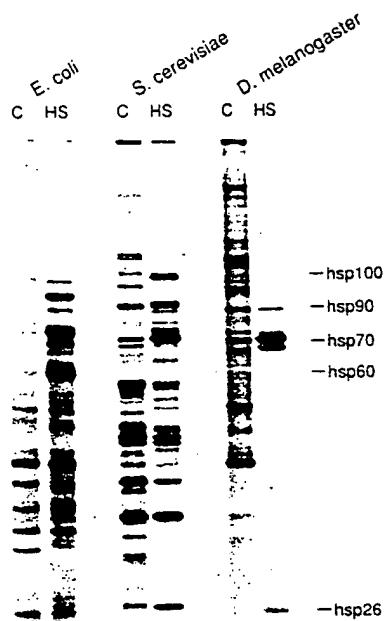
Figure 1   Induction of heat-shock proteins. Logarithmically growing cells were pulse-labeled with ^{3}H-leucine either while growing at normal temperatures (*E. coli*, 37°C; *S. cerevisiae*, 25°C; *D. melanogaster*, 25°C) or following a shift to slightly elevated temperatures (*E. coli*, 50°C, 10 min; *S. cerevisiae*, 39°C, 20 min; *D. melanogaster*, 36.5°C, 45 min). Total cellular proteins were extracted, separated on a 10% SDS-polyacrylamide gel, and visualized by fluorography. Figure from (151a).

Table 1   Major heat-shock protein families

Table 1 Major heat-shock protein families

Protein family	Family members	Monomer size (kd)	Eukaryotic location	Stress functions	Other comments
hsp100	hsp104, ClpA, ClpB, ClpC, ClpX	80–110 kd 46 kd (ClpX)	Cytoplasm, nucleus, nucleolus, (hsp104) Chloroplast (cpC)	• Extreme heat tolerance (yeast hsp104, _E. coli_ ClpB) • Ethanol tolerance, long-term spore viability (yeast hsp104) • Regulation of ClpP protease (_E. coli_ ClpA and ClpX)	• Assembles into hexameric (yeast hsp104, _E. coli_ ClpA) or tetrameric (_E. coli_ ClpB) particles • Assembly requires adenine nucleotide binding (yeast hsp104, _E. coli_ ClpA) • Association with ClpP makes protease ATP-dependent (_E. coli_ ClpA) • ATPase activity (_E. coli_ ClpA and ClpB, yeast hsp104)
hsp90	hsp82, grp94, HtpG	82–96	Cytoplasm, ER, nucleus	• Essential for viability (yeast hsp/c82) • Higher concentration required for growth at higher temperatures (yeast)	• Dimer • Associates with immunophilins, hsp70, actin, tubulin, steroid hormone receptors, _src_-related kinases • Promotes activity of casein kinase II, steroid hormone receptors, _v-src_ kinase; represses receptors and _src_ kinases while associated • May have general chaperoning activity • ATPase activity
Lon	Lon	94	(Prokaryotic)	• ATP-dependent proteolysis of abnormal proteins	• Tetramer • Promotes ATP-dependent degradation of certain normal proteins (for example SulA, RcsA) • ATPase activity

Table 1 *(continued)*

Protein family	Family members	Monomer size (kd)	Eukaryotic location	Stress functions	Other comments
hsp70	DnaK, grp78, hsc70, BiP, Kar2, Ssa, Ssb, Ssc	67–76	Different members occupy different compartments: Cytoplasm, nucleus, mitochondria, chloroplasts, ER	• Molecular chaperone required for protein assembly, secretion, import into ER and organelles • Heat-inducible proteins required for growth at moderately high temperatures (yeast, *E. coli*) • Promotes survival at extreme temperatures (yeast, Drosophila, mammals) • Facilitates proteolysis of abnormal proteins (*E. coli*) • Negatively regulates hsp synthesis (*E. coli*, yeast, Drosophila)	• Monomer, dimer, higher-order oligomerization may serve to inactivate protein • Binds unfolded proteins and peptides • Cooperates with hsp60 in mitochondrial protein assembly and the folding of denatured substrates • Association with DnaJ and GrpE influences interaction with substrates and promotes ATP hydrolysis • Associates with hsp90 • ATPase activity
hsp60	GroEL, hsp65, cpn60, Rubisco binding protein	58–65	Mitochondria, chloroplasts	• Molecular chaperone that facilitates folding of monomeric proteins and assembly of oligomeric protein complexes • Higher concentration required for growth at higher temperatures (*E. coli*) • May facilitate proteolysis of abnormal proteins (*E. coli*)	• Forms seven-membered rings. In most cases, two rings assemble to form a "double-doughnut" • ATP hydrolysis and substrate release stimulated by binding of hsp10 (GroES) • ATPase activity

Name	Members	Size (kDa)	Location	Function	Properties
DnaJ	Scj1, Ydj1, Sis1, Hdj1, Sec63, ResA	40–100	Cytoplasm, membranes	• Functions with hsp70 to promote reactivation of stress-damaged substrates • Facilitates proteolysis of abnormal proteins (*E. coli*) • Negatively regulates hsp synthesis (*E. coli*)	• Dimer • Associates with other hsps, including hsp70, GrpE, hsp90 • Binds to both folded and unfolded protein substrates • Helps target DnaK to various substrates
TF55	Tcp1, TRiC, thermosome	55–60	Cytoplasm	• Required for viability (yeast) • Induction of TF55 is coincident with acquisition of thermotolerance (archaebacteria)	• Eight and nine-membered rings • Eukaryotic and some archaebacterial TRiCs are hetero-oligomeric • Binds unfolded proteins • Promotes proper folding of tubulin, actin, luciferase • Not heat-inducible in yeast or mammals
FKBPs	FKBP12, FKBP13, FKBP25, FKBP59, Fpr1, Nep1	12–60	Cytoplasm, ER	Unknown	• FKBP59 is Hsp56 • Peptidyl prolyl isomerase (PPI) • Binds immunosuppressants FK506 and rapamycin • FKBP-drug complex binds calcineurin • Hsp56, together with hsp70 and hsp90, associates with the steroid receptors and p60^{src}
cyclophilins	cyclophilin A (Cph, Cyp1, Cpr1), cyclophilin B (Crg, Cyp2, Crp2), cyclophilin C (Scc3, Cpr4, Cpr3)	11–40	Cytoplasm, ER, mitochondria	• Heat-inducible proteins (Cyp1, Cyp2) promote survival at high temperatures (yeast)	• Peptidyl prolyl isomerase (PPI) • Binds immunosuppressant cyclosporin A and drug complex binds calcineurin

Table 1 (*continued*)

Protein family	Family members	Monomer size (kd)	Eukaryotic location	Stress functions	Other comments
hsp27	hsp18, hsp22, hsp23, hsp26, hsp27	12–40	Cytoplasm, nucleus, chloroplast, ER	• Contributes to thermotolerance (mammals)	• Forms large (20S) particles • Structurally related to α-crystallin • Particularly abundant in plants after heat shock • May have general chaperone activity
ClpP	clpP	20–30	Chloroplasts	• Proteolysis of abnormal proteins (*E. coli*)	• Dodecameric, cylindrical particle that resembles eukaryotic proteasome • Serine protease • Associates with either ClpA or ClpX to promote cleavage of particular substrates in *E. coli*
GrpE	grpE	20	Unknown	• Functions with hsp70 to promote reactivation of stress-damaged substrates • Facilitates proteolysis of abnormal proteins (*E. coli*) • Negatively regulates hsp synthesis (*E. coli*)	• May promote release of substrates bound to DnaK by facilitating ADP/ATP exchange

hsp10	GroES, cpn10	9–12	Mitochondria, chloroplasts	• Stimulates hsp60 functions • Seven-membered ring shaped particle • Associates with hsp60 and promotes substrate release
ubiquitin	very highly conserved protein found in all eukaryotes	8	Cytoplasm, nucleus	• Targets abnormal proteins for degradation by the 26S protease • Polyprotein cleaved into monomers after synthesis • Covalently attached through C-terminus to proteolytic substrate • Some ubiquitin-conjugating enzymes (E2s) are also heat-inducible

members, and the constitutive proteins are frequently very abundant. Their roles in normal cellular physiology have recently been the subject of several excellent reviews (56, 79, 98, 273). This review focuses on how the proteins in the major hsp families help organisms survive during conditions of stress.

Historically, three observations suggested that hsps protect cells and organisms from stress. First, their induction has the character of an emergency response, being extremely rapid and very strong. For example, in Drosophila there are five copies of the *HSP70* gene per haploid genome, and regulation at the levels of transcription, translation, RNA processing, and RNA stability work together to produce a greater than 1,000-fold induction of the protein upon heat shock (126, 146, 151, 161, 189, 194, 199, 285, 288). The complexity of the regulatory mechanisms in place for hsp70 suggests that it is vitally important for cells to rapidly accumulate this protein during times of stress. Hsp induction in other organisms, while not quite as extreme as in Drosophila, shares these characteristics of swiftness and specificity.

Second, hsps are induced at very different temperatures in different organisms, but. in each case, the induction temperature reflects stress conditions for the organism. For example, thermophilic bacteria induce heat-shock proteins when shifted from 95°C to 105°C (202, 261), and arctic fishes induce them when shifted from 0°C to 5–10°C (B Maresca, personal communication). Organisms induce hsp synthesis when their temperature increases above that which is normal for them, rather than at a universal temperature threshold.

Finally, and most compellingly, the induction of hsps correlates with the induction of tolerance to extreme heat in a wide variety of cells and organisms (142, 180, 188, 218). Hsp induction also correlates with induced tolerance to other stressful treatments (e.g. high concentrations of ethanol, arsenite, or heavy metals) (147, 188, 220, 250). Moreover, the correlation is as strong for developmentally regulated inductions as it is for stress-mediated inductions. In yeast, for example, spores and stationary phase cells constitutively express hsps and have high basal levels of thermotolerance (205, 220, 224). Similarly, germinating wheat seeds constitutively express hsps and have high intrinsic thermotolerance (1). Conversely, developmental stages in which organisms are unable to express hsps, such as the early embryonic stages of Drosophila (53, 93), snails (20), sea urchins (230), Xenopus (99), and mammals (177, 269) are characterized by unusual thermosensitivity (24).

The roles of individual hsps in stress protection are beginning to be dissected. In *Saccharomyces cerevisiae*, for example, some hsps are required for growth at temperatures near the upper end of the normal growth range (e.g. hsp70) (42), others for long-term survival at moderately high temper-

atures (e.g. ubiquitin) (62), and still others for tolerance to extreme temperatures (e.g. Hsp104) (218). Furthermore, different organisms use different hsps to respond to similar levels of stress. For example, tolerance to extreme stress depends largely upon Hsp104 in yeast, upon hsp70 in Drosophila, and upon hsp70, hsp27, and perhaps hsp110 in mammals (133, 142, 218, 240). Understanding the reasons for this diversity in tolerance strategies will depend on elucidating how individual hsps function in protecting cells from stress.

The common signal for hsp induction appears to be the presence of "abnormal" proteins. Treatments that efficiently induce hsp synthesis in vivo also damage or denature proteins in vitro. Thus, exposure to high concentrations of ethanol, sodium arsenite, or heavy metal ions all trigger a heat shock-like response (142, 188). Amino-acid analogs and puromycin, both of which result in abnormal proteins, are efficient inducers of hsps (83a, 204). Moreover, intracellular accumulation of foreign proteins, denatured proteins, or proteins containing structurally destabilizing amino-acid substitutions induces hsp synthesis in a variety of cell types (3, 82, 196).

There are at least two general ways in which hsps might help cells to cope with stress-induced damage to polypeptides. First, hsps could promote degradation of abnormal proteins. In fact, some hsps have intrinsic proteolytic activities, and others serve auxiliary roles in proteolysis. Second, hsps could reactivate stress-damaged proteins. Several hsps are known to function as "molecular chaperones," preventing the aggregation and promoting the proper refolding of denatured proteins. The features that usher some stress-damaged proteins along the degradation pathway and others along the renaturation pathway are not currently understood.

In the sections that follow, we present an overview of current knowledge of hsp function in the cellular response to stress. We focus first on those hsps involved in degrading damaged polypeptides and later on those involved in salvaging them.

TURNOVER OF ABERRANT PROTEINS IN E. COLI

The half-lives of most cellular proteins in E. coli are long (t_{1_2} = 5–20 h) compared to the short generation time of the organism (30 min). However, abnormal proteins (amino-acid analog-containing proteins, truncated polypeptides and some mutant proteins) are recognized and degraded very rapidly (t_{1_2} = 1–20 min). Rapid degradation of damaged proteins reduces the possibility of deleterious interactions between these polypeptides and functional proteins, prevents accumulation of insoluble protein aggregates, and releases the amino acids contained in nonfunctional polypeptides for synthesis of new proteins.

Much of the cytosolic degradation of abnormal proteins in E. coli is

accomplished by two proteases, Lon and Clp. Although these enzymes do
not share sequence similarity, they do have common features. Both employ
the energy of ATP hydrolysis in protein degradation, and both are consti-
tutively expressed and induced two- to fourfold by heat (82, 129, 201).
They are currently the best candidates for controlling the degradation of
stress-damaged proteins.

The Lon Protease

Lon (also known as protease La) functions as an ATP-dependent protease
composed of four identical 783 amino-acid subunits. Inhibitor studies and
mutational analysis of the putative active site suggest that Lon defines a
novel class of serine proteases (2, 37, 270, 271). A fairly detailed mechanism
for the activity of this protease has been proposed (see (83, 158), for
review). Interestingly, the way in which ATP binding and hydrolysis are
coupled to the release of proteolytic substrates from Lon is strikingly similar
to the way in which proteins engaged in chaperoning activities are thought
to release their substrates (158).

Biologically, Lon serves a dual role. In its "specific" role, Lon catalyzes
the turnover of several key regulatory proteins (for review, see (88, 91)),
the best-studied being SulA and RcsA. Normally, SulA synthesis increases
in response to DNA damage, and it inhibits cell septation. Once the
damage has been repaired, synthesis of SulA is repressed, and the protein
is rapidly degraded by Lon, allowing septation to proceed. In lon mutants,
SulA levels remain high, and cells become filamentous and die in response
to UV irradiation and other DNA-damaging treatments. lon mutants also
have a characteristic mucoid appearance, resulting from over-expression
of the cps genes necessary for capsule synthesis. The level of the positive
regulator of these genes, RcsA, is normally controlled by rapid degradation
by Lon.

In its "general" role, Lon promotes the degradation of abnormal proteins.
Its substrates include canavanine-containing proteins, puromycyl peptides,
and many missense proteins and nonsense fragments (for review, see (88,
91)). In fact, it is estimated from studies with canavanine-containing proteins
that Lon is responsible for roughly half of the turnover of abnormal proteins
in E. coli.

An interesting paradox exists with respect to substrate recognition by the
Lon protease. While the protease shows great specificity in catalyzing the
turnover of "specific" substrates such as SulA and RcsA, it appears to be
able to recognize almost any protein once the protein has been denatured.

Lon's preference for abnormal protein substrates, together with the fact that
it is induced by heat and other stresses, has led to the hypothesis that Lon helps
to eliminate proteins that become denatured during stress. Surprisingly,

however, since *Lon* mutants are neither temperature-sensitive nor less thermotolerant than wild-type cells (S Gottesman, personal communication), the role of Lon in stress-tolerance is yet to be determined.

DnaK, DnaJ, GrpE, GroEL, and GroES

In addition to Lon, other hsps are important for the ability of *E. coli* to degrade abnormal proteins. In *lon⁻* as well as *lon⁺* backgrounds, mutation of the sigma factor that controls heat-shock gene expression (σ^{32}) results not only in decreased expression of hsps, but also in diminished proteolysis of some abnormal proteins (7). Moreover, individual mutations in several heat-shock genes, *dnaK*, *dnaJ*, *grpE*, and *groEL*, cause substantial defects in the energy-dependent turnover of abnormal proteins (118, 249).

Recent work demonstrates a direct interaction between some proteolytic substrates, Lon and other hsps. DnaK, GrpE and Lon co-immunoprecipitate with a rapidly degraded mutant variant of alkaline phosphatase (234a). Furthermore, affinity chromatography with an abnormal chimeric protein yields Lon, DnaK, GrpE, and GroEL (235). In the latter study, GroEL and DnaK appeared to bind directly to the proteolytic substrate. Whether Lon binds independently to these proteolytic substrates or interacts with the hsps in a larger complex is not known.

Hsps play a larger role in proteolysis than can be accounted for solely by cooperation with Lon. Hsp mutants are more defective than Lon mutants in the turnover of abnormal proteins, and overexpression of hsps enhances proteolysis of puromycyl fragments even in *lon⁻* strains (7, 81, 118, 249). Furthermore, DnaK, DnaJ, and GrpE are required for the turnover of certain proteins that are not Lon substrates (249). Perhaps hsps cooperate with several different proteases to enhance the degradation of aberrant polypeptides.

Hsps could function completely independently of proteolytic complexes and still have a significant impact on the flux of substrates through the various proteolytic pathways of the cell. *dnaK*, *dnaJ*, *groEL*, *groES*, and *grpE* mutants all accumulate over-expressed RNA polymerase subunits as insoluble aggregates. These same polymerase subunits are rapidly degraded in wild-type cells. Perhaps the hsps employ their anti-aggregation activities (see chaperone section below) to prevent the subunits from accumulating in a degradation-resistant form (A Gragerov, personal communication).

The studies described above suggest two different ways that hsps like DnaK, DnaJ, GrpE, GroEL, and GroES might promote intracellular proteolysis. Both models employ the ability of hsps to function as "chaperones" by binding to denatured proteins and preventing their aggregation. On one hand, hsps could interact directly with protease complexes, either serving to maintain the substrate in a protease-sensitive conformation or stabilizing

a more active conformation of the protease itself. In fact, substrates bound to GroEL in vitro are maintained in a conformation that is particularly sensitive to proteases (157). Alternatively, hsps could function separately from proteases, preventing aggregation of unfolded proteins, increasing the concentration of susceptible substrates, and thereby indirectly promoting proteolysis.

The Clp Protease

Experiments assessing the breakdown of amino-acid analog-containing proteins suggest that approximately 15% of turnover of abnormal proteins in *E. coli* is accomplished by the Clp protease (also known as protease Ti) (117). Clp is a 750-kd hetero-oligomer composed of two unrelated types of subunits, ClpP and ClpA (for review, see (158)). The 21.5-kd ClpP protein assembles as a dodecameric, cylindrical particle and forms the catalytic core of the protease. By itself, ClpP functions in vitro as an ATP-independent serine protease capable of degrading peptides and short polypeptides. However, the ClpP particle is unable to catalyze the turnover of larger protein substrates unless it is associated with ClpA, a protein that serves as an ATP-dependent regulator of ClpP. ClpA is an 83-kd ATPase that hexamerizes in the presence of ATP prior to association with the dodecameric ClpP. ATP is required both for the assembly of ClpA with ClpP and for substrate cleavage by the Clp protease.

Unlike Lon, where native substrates are known, the normal physiological role of Clp has been difficult to determine. Cells carrying mutations in either *clpP* or *clpA* show decreased degradation of ClpA-β-galactosidase fusion proteins, so it is thought that Clp is involved in the turnover of its own regulatory subunit (90). ClpA mutants are also defective in the degradation of test substrates containing "destabilizing" amino acids at their N-termini (258). In eukaryotes, these substrates are ubiquitin-tagged and then degraded by the 26S protease (see below). There are striking structural similarities between the prokaryotic Clp protease and the eukaryotic 26S protease (reviewed in (158)). Both are cylindrical particles composed of stacked six-membered rings, and both associate with ATP-dependent regulatory components. That they also appear to recognize some of the same substrates suggests that the prokaryotic and eukaryotic proteases are functional homologs. However, since the physiological relevance of "N-end rule" degradation is not yet appreciated (12), this observation does not elucidate the biological function of Clp in *E. coli*.

ClpP and ClpA belong to distinct, highly conserved protein families containing both prokaryotic and eukaryotic members (92, 160). The ClpP family includes several plant chloroplast proteins that share ~40% amino-acid identity and an additional 30% similarity to *E. coli* ClpP, and ClpP

antiserum reacts with proteins from a variety of eukaryotes and prokaryotes. ClpA-like proteins (the Clp family) have their greatest similarity in two large regions (200–300 amino acids), each centered around an ATP-binding consensus sequence (92, 195, 241, 242). The level of amino-acid homology in these regions is remarkable: 50–80% identity and an additional 20–40% similarity. This protein family is divided into subfamilies based upon the organization of the nucleotide-binding domains. In the ClpA subfamily, the domains are almost contiguous. In the ClpB subfamily they are separated by a large (~120 amino acids) nonconserved spacer, and in the ClpC subfamily a smaller (60–70 amino acids) spacer is interposed.

The ClpA subfamily includes the ClpA protein from *E. coli*, whose function as a proteolytic regulator has been discussed. ClpA proteins are not heat-inducible, and *E. coli* strains carrying *clpA* mutations are neither temperature-sensitive for growth nor less thermotolerant than wild-type cells (S Gottesman, personal communication).

The ClpB subfamily currently includes proteins from bacteria, yeast, trypanosomes, and plants. All are strongly heat-inducible. Immunological and sequence data indicate that the well-known high molecular weight hsps from mammals and yeast are members of this group. Thus, the Clp family is also referred to as the hsp100 family (Figure 2; 195). Two proteins from the ClpB subfamily, *E. coli* ClpB and *S. cerevisiae* Hsp104, have been studied in some detail (280; DA Parsell & S Lindquist, unpublished data). Like ClpA, both are ATPases, although they do not appear to interact with ClpP or to promote proteolysis in vitro (280; M Maurizi, personal communication). Moreover, *hsp104* and *clpB* mutants have no detectable proteolytic defects (197; S Gottesman personal communication). In yeast, for example, neither the turnover of short-lived normal proteins nor canavanine-containing proteins is affected by disruption of *HSP104*. Furthermore, unlike ubiquitin pathway mutants, *hsp104* mutants are not hyper-sensitive to amino-acid analogs. Mutations in *HSP104* and *clpB* do, however, compromise tolerance to extreme temperatures (see Hsp100 section below) (218, 242).

The ClpC subfamily includes proteins from both bacteria (*Mycobacterium leprae*) and plants. The eukaryotic members of this group contain sequences at their N-termini that target them to chloroplasts, where ClpP-like proteins are synthesized. It seems likely that eukaryotic ClpC proteins associate with chloroplast ClpP homologs and play a role in organellar protein turnover (241).

The newly described ClpX and ClpY proteins of *E. coli* are truncated members of the hsp100 family. They lack the leader, spacer, and first nucleotide-binding domain regions of other family members, and have only limited homology to the other proteins in the second nucleotide-binding domain and the carboxy-terminal regions (89). Nevertheless, like ClpA, the

Hsp100

Hsp90

Hsp70

Hsp60

TCP/TF55

Variance

Hsp10

Plant/yeast smhsps

Animal smhsps/α-crystallins

Amino Acid Position
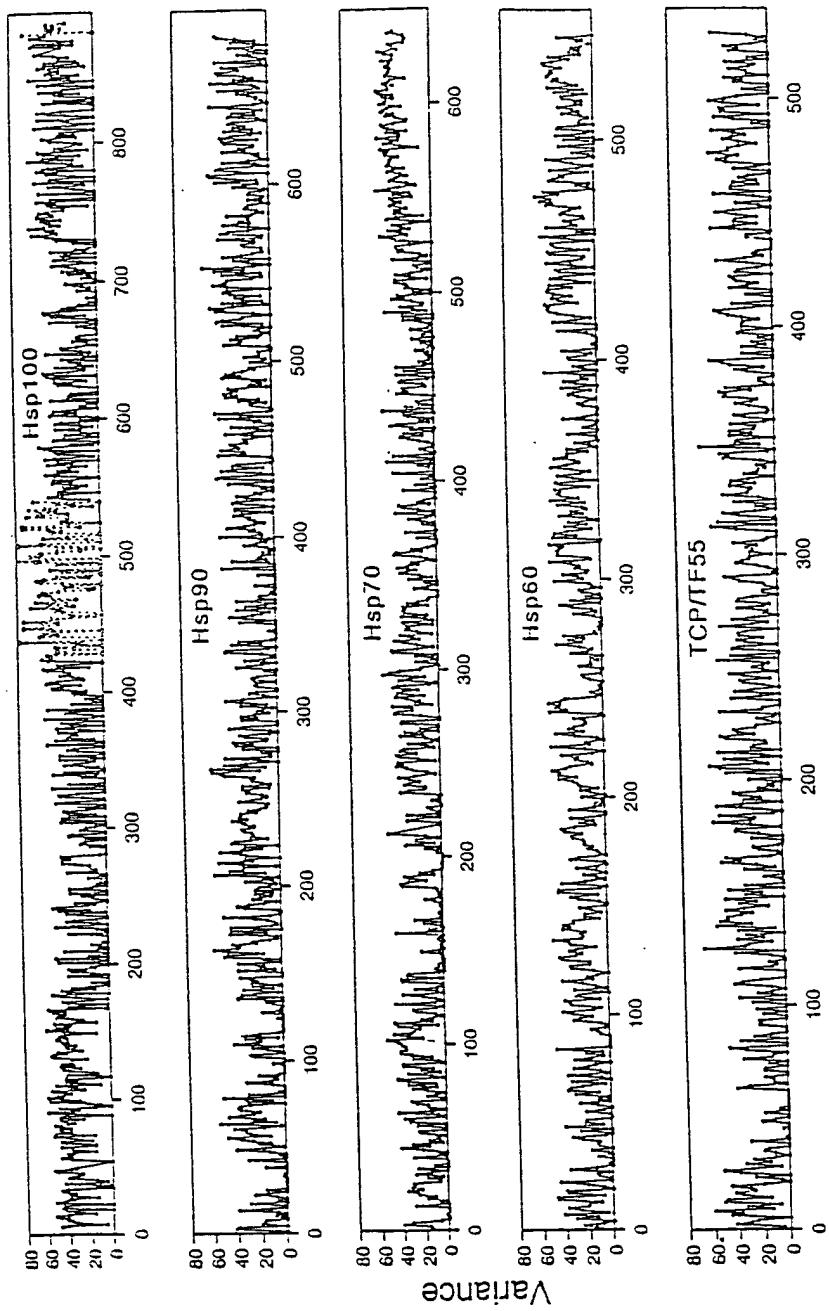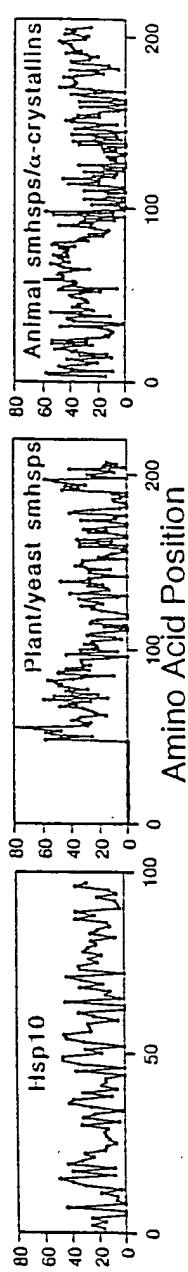
Figure 2 Sequence homology within the major heat-shock protein families. Members of the major hsp families whose sequences were represented in the SwissProt database were aligned using the PredictProtein program (221). A "variance" score was assigned to each residue position based on the similiarity of the amino acids present in different family members at that position. A variance of zero indicates that a particular residue was identical in all members of the family. Therefore, the most conserved regions of these proteins have a large number of points on or near the X-axis. The program does not assign any penalty for gaps. Specific information about the alignment program can be obtained via electronic mail: Predict-Help @EMBL-Heidelberg.de.

For most hsp families, many proteins from a wide variety of phylogenetic groups and several subcellular compartments were compared. An exception was the hsp100 family, for which only five members are represented in the SwissProt database. (For a more detailed comparison of this family, see 92). The small number of sequences for the hsp100 alignment presents a problem because some contain large gaps in the spacer region or are missing C-terminal residues. The variance scores in these regions are not informative and have been de-emphasized using open symbols and dashed lines.

For the hsp90 family, 24 sequences were compared, including proteins from bacteria, yeast, and animals. For the hsp70 and hsp60 families, 83 and 37 proteins, respectively, were compared, including proteins from bacteria, yeast, plants, and animals. For the TCP/TF55 family nine sequences were compared, including the TF55 sequence from *Sulfolobus shibatae* and TCP sequences from yeast, Drosophila, and mammals. For the hsp10 family, nineteen sequences were aligned from bacteria, plants, and mammals. Because of the very limited homolgy among the small hsps, this family was divided into two groups based upon amino-acid similarity. Fifteen yeast and plant smhsps were compared to each other. Similarly, 58 proteins, including α-crystallins from many species, and smhsps from mammals, Drosophila, and *C. elegans* were compared. The plant smhsps are missing a stretch of 48 N-terminal residues, so the variance scores in this region have been de-emphasized with open symbols.

E. coli ClpX protein regulates the proteolytic activity of ClpP. In vitro and in vivo the ClpX/ClpP complex degrades the lambda O protein. Some rapidly degraded *vir* mutants of bacteriophage Mu also appear to be substrates of this complex. ClpX is a heat-inducible protein synthesized from the same operon as ClpP, but, as is the case for *clpP*, mutations in *clpX* do not render the cell sensitive to high temperatures.

The role of the Clp protease in the turnover of stress-damaged proteins is unclear. Although the synthesis of the catalytic subunit increases about twofold upon heat shock (129), ClpP mutants are not temperature-sensitive for growth and do not have increased susceptibility to extreme stress. Similarly, no stress-related phenotypes have been observed in regulatory subunits (ClpA and ClpX) mutants. Proteins related to the regulatory subunits (e.g. Hsp 104) have profound effects on stress tolerance, but the relationship between their tolerance functions and the turnover of abnormal protein is not apparent.

The DegP Protease

One heat-inducible protease plays an important role in helping *E. coli* cope with stress-induced damage in the periplasm. DegP (HtrA) is a 48-kd periplasmic serine protease that is strongly heat-inducible and essential for growth at high temperatures (152, 247). *DegP* mutations prevent the turnover of periplasmic, but not cytoplasmic, fusion proteins (153, 246), suggesting that DegP promotes the turnover of abnormal proteins in the periplasm. Thus, although much less is known about DegP than about Lon and Clp, it currently provides the best evidence for the biological importance of proteolysis in stress tolerance.

TURNOVER OF ABERRANT PROTEINS IN EUKARYOTES

Many proteins degraded in the eukaryotic cytosol are proteolyzed by the ubiquitin system (for review, see 61, 108, 114). This complex set of enzymes degrades not only abnormal proteins, but also a number of important short-lived regulatory proteins, including the plant phytochrome protein Pfr, mammalian p53, amphibian cyclin and yeast MATα2 (80, 109, 222, 233). Ubiquitin is a small (76-amino acid) abundant protein that is highly conserved in eukaryotes. Its role in proteolysis is to serve as a tag, marking substrates to be degraded. First, ubiquitin is activated by attachment to an E1 enzyme (ubiquitin activating enzyme) in an ATP-dependent reaction. Activated ubiquitin is then transferred to one of a set of E2 proteins (ubiquitin conjugating enzymes), and the E2 enzyme catalyzes the transfer of ubiquitin to a lysine residue on the protein to be degraded. For some substrates, E2s catalyze ubiquitin transfer directly, while for others the action of an additional

enzyme, an E3 (ubiquitin protein ligase), is required. Additional ubiquitin moieties are subsequently attached to the first, forming a branched array that targets the protein for degradation by a multi-subunit ATP-dependent protease (26S protease).

Ubiquitin-dependent protein degradation functions in the stress response in two ways. First, some of the key reactions in this pathway are catalyzed by heat-inducible proteins (see below). Second, the ubiquitin system appears to be responsible for much of the turnover of eukaryotic stress-damaged polypeptides. When cultured cells are subjected to a sudden increase in temperature, a burst of degradation of normally long-lived proteins is observed (193). The increase in proteolysis coincides with a reduction in free and histone-conjugated ubiquitin and an increase in multi-ubiquitin-protein conjugates (193). Furthermore, a mutant cell line containing a temperature-sensitive E1 enzyme (ts85) does not respond to temperature upshift with increased proteolytic activity and cannot grow at high temperatures.

Ubiquitin

In a wide variety of eukaryotes, ubiquitin is synthesized as a polyprotein, which is subsequently processed into individual ubiquitin units (19, 140, 190). In every case tested, these poly-proteins are heat-inducible. Genetic evidence demonstrates that raising the intracellular concentration of free ubiquitin is an important part of the cellular response to stress. Although *S. cerevisiae* polyubiquitin (*UBI4*) mutants grow at wild-type rates over the normal range of growth temperatures for yeast, they are more sensitive than wild-type cells to prolonged incubations at temperatures just beyond the growth range, to starvation, and to amino-acid analogs (62). Ubi4–deficient cells, however, are not impaired in their ability to withstand short incubations at extreme temperatures.

In *S. cerevisiae*, the polyubiquitin gene *UBI4* provides the additional ubiquitin required by stressed yeast cells. Under normal conditions, ubiquitin is synthesized by the *UBI1*, *UBI2*, and *UBI3* genes. These genes each encode fusion proteins, with an N-terminal ubiquitin and a C-terminal ribosomal protein (60), that are precisely cleaved after synthesis. The transient attachment of ubiquitin to the ribosomal proteins seems to facilitate ribosome assembly, suggesting that ubiquitin may function as a molecular chaperone specific for the protein to which it is fused (60). Ubiquitin's chaperone activities may not be limited to ribosomal proteins, as several other proteins with ubiquitin-like sequences at their N-termini have been discovered in vertebrates (8, 96, 171, 259). Interestingly, the fusion of ubiquitin coding sequences to the N-termini of some foreign proteins can significantly enhance their expression in *E. coli* and yeast (31, 54). The reason for this is not yet understood, but it is possible that ubiquitin is an

hsp that plays a role both in degrading proteins and in chaperoning protein folding and assembly.

Ubiquitin-Conjugating Enzymes (E2s)

The ubiquitin-conjugating enzymes (E2s) catalyze the transfer of ubiquitin to proteolytic substrates. Over 30 genes, from a variety of organisms, have been shown to encode E2 enzymes (see 114 for review). *S. cerevisiae* contains an essential subfamily of E2 genes, two of which, *UBC4* and *UBC5*, encode nearly identical heat-inducible proteins (232). Ubc4/5 function is crucial for cell survival under conditions of stress. The *ubc4ubc5* double mutant is inviable at elevated temperatures and is very sensitive to amino-acid analogs such as canavanine. In addition to being induced by high temperatures, expression of *UBC4* and *UBC5* increases upon exposure to cadmium (116). The *ubc4ubc5* double mutant is hypersensitive to cadmium, suggesting that these proteins are also important for the turnover of cadmium-damaged proteins.

The Ubc7 protein is another E2 enzyme that probably plays a role in eliminating cadmium-damaged proteins. *UBC7* is strongly induced when cells are exposed to cadmium, and *ubc7* mutants are hypersensitive to cadmium (116). Unlike *UBC4/5*, the expression of *UBC7* does not increase with heat shock, and *UBC7* mutants are not temperature-sensitive.

Ubiquitin-Protein Ligases (E3s)

Targeting certain substrates for ubiquitin-dependent turnover requires both an E2 and an E3 enzyme. To date, the yeast Ubr1 protein is the only E3 enzyme whose function has been examined genetically (12). Ubr1 functions as the recognition component for substrates containing "destabilizing" N-terminal residues. *UBR1* is not heat-inducible and *ubr1* mutants are not more sensitive than wild-type cells to heat or other stresses. These observations suggest that N-end rule recognition does not play an important role in stress tolerance. Other as yet undefined E3s may play such a role. Alternatively, eukaryotic hsps with chaperoning functions (see below) might serve in an E3-like capacity, targeting stress-damaged proteins for ubiquitin-dependent degradation (114).

The 26S Protease

The enzyme responsible for degrading ubiquitin-tagged substrates is a large, multi-subunit ATP-dependent protease (26S protease). This complex includes the 20S multi-catalytic proteasome and several other proteins (MW 35–110 kd) (for review see (108, 209)). One of the high molecular weight subunits (S4) of the mammalian 26S protease belongs to a newly described family of ATPases (52, 57, 70). S4 also shares weak homology with the *E. coli* ClpA protein in the first ATP-binding region, providing additional

evidence that the 26S protease may represent a functional homolog of the bacterial Clp enzyme (52).

Although most of its components are not heat-inducible, the 26S protease is clearly important for stress tolerance. Yeast strains carrying a missense mutation in the proteasome subunit, *pre1*, show increased sensitivity to both high temperatures and amino-acid analogs (100).

Proteolysis in the Lysosome

More than 90% of long-lived eukaryotic proteins, and a large fraction of short-lived ones, are degraded in a nonselective manner in the lysosome (see (18) for review). In cultured cells, however, the stress of serum withdrawal doubles the rate of proteolysis of certain long-lived proteins that contain a sequence similar to the consensus (K-F-E-R-Q). A constitutive member of the hsp70 family binds to this sequence, and may help during starvation to target proteins containing this sequence to the lysosome (48). Lysosomal proteolysis during starvation may also depend on the ubiquitin pathway. Starved cells that are deficient in ubiquitin pathway components have reduced rates of lysosomal proteolysis (94), and anti-ubiquitin immunogold particles decorate lysosomes in electron micrographs (51, 229).

Proteolysis in the Endoplasmic Reticulum

Although the enzymes responsible for protein turnover in the ER are not known, aberrant polypeptides are rapidly proteolyzed in this compartment (125). A member of the hsp70 family, BiP, binds to unassembled subunits of multimeric ER proteins and may be involved in their proteolysis. BiP is induced by heat shock and by unfolded proteins in the ER (35, 128, 176, 211). However, experiments have yet to establish a connection between the binding of abnormal proteins by BiP and degradation.

The ubiquitin system may also be involved in protein turnover in the ER. An E2 enzyme, ubc6, localizes to the ER membrane, and genetic studies suggest it mediates the selective turnover of ER membrane proteins (115). The proteasome associates with the ER upon purification (127), and some ER membrane proteins appear to be ubiquitinated (256), providing additional links between ubiquitin-dependent proteolysis and ER membrane proteins.

THE CHAPERONE FUNCTIONS OF HSPS

Protein Folding

Hsps are diverse in size and oligomeric composition, yet several have a common functional theme—modulating the folding and unfolding of other proteins and facilitating assembly and disassembly of multisubunit complexes. Because a key element of this function involves preventing improper

associations, these hsps are often called molecular chaperones (55). A central feature of this function is that chaperones do not impart steric information to other proteins. Rather, the information that drives the acquisition of native structure is contained within the primary amino-acid sequence of the protein itself. Chaperones simply facilitate the self-directed folding process.

The relationship between normal protein folding and the off-pathway reactions that occur as a result of heat stress warrants a brief description of the forces that govern protein folding (see (68, 98, 122, 123, 185) for detailed treatments).

Electrostatic interactions, hydrogen bonding, and van der Waals interactions all play important roles in stabilizing the native structures of proteins, but the dominant force driving folding is the hydrophobic effect. In aqueous environments, hydrophobic side chains cause surrounding water molecules to adopt an ordered, lattice-like structure that is entropically very unfavorable. Therefore, the first step in the folding of a protein domain, occurring on a millisecond timescale, involves the collapse of hydrophobic side chains into the "core" of the molecule. This collapse allows these sidechains to interact with each other and shields them from the surrounding aqueous environment. On the same time scale, units of secondary structure form, primarily α helices and β sheets. At this stage, the protein has a compact structure, but lacks stable tertiary interactions and is thought to resemble the "molten globule" state observed in some proteins under very specific, mild denaturation conditions. The transition from this structure to the final native structure generally requires 50 milliseconds to a few minutes (68). While the many interactions among side chains in the native structure are enthalpically favorable, the conformational restrictions imposed on the polypeptide chain make this state entropically less favorable than the unfolded form. The net effect of these opposing forces on protein folding is a very small free energy of stabilization, for most proteins on the order of 5–15 kcal/mol.

Although the primary amino-acid sequence contains all the information required to generate the final folded structure of a protein, the highly concentrated environment of the cell can make this structure difficult to attain. At several points during the "life" of a protein, surfaces that are buried in the native structure are transiently exposed and are susceptible to unproductive, off-pathway associations with other proteins. During synthesis, nascent chains are susceptible to such associations before the remainder of the protein has emerged from the ribosome. After synthesis is complete, individual subunits of oligomeric proteins expose surfaces that will be hidden once they assemble with their partners, and proteins destined for other compartments traverse the cytosol and adopt a loosened conformation in order to cross organellar membranes. The role of molecular chaperones in these processes is to bind and transiently sequester interactive protein surfaces (56, 98).

In considering the effects of high temperatures on protein structure, two problems are salient. First, intermediates in the initial folding process are particularly susceptible to off-pathway interactions and aggregation. For example, a variety of foreign proteins aggregate and form inclusion bodies when expressed at high levels in *E. coli*. In most cases, productive folding is greatly increased when the growth temperature is lowered (223). Once these proteins are properly folded, they are not particularly sensitive to denaturation and aggregation. Thus, nascent chains and folding intermediates are particularly sensitive to high temperatures. The same susceptibility presumably applies to the nascent chains of fully synthesized proteins as they traverse intracellular membranes.

Second, once a protein is folded, localized, and properly associated with its oligomeric partners, it remains susceptible to denaturation by extreme temperatures and other forms of stress. In vitro studies suggest that the melting temperatures of many protein domains are well within the range of temperatures achieved during a physiological heat stress. In the highly concentrated protein environment of the cell, hydrophobic side chains exposed as a protein unfolds will interact with other unfolded proteins or with hydrophobic surfaces on proteins that are in the process of synthesis, assembly, or transport. Thus, a relatively small amount of denaturation may evoke disaster as unfolded protein domains pull covalently attached folded domains and oligomeric partners into aggregates.

To prevent such disasters, molecular chaperones must be present at very high concentrations so that unfolded proteins will be more likely to interact with them than with other reactive surfaces. Accordingly, as temperatures begin to increase, a wide variety of regulatory mechanisms are employed to ensure that chaperones are induced as rapidly as possible. This induction is quite selective, however, and many chaperones are not induced by heat at all. One explanation for this selectivity is that the major heat-induced chaperones are generalists. In fact, the hsp70 and hsp60 proteins appear to recognize simple structural motifs that are shared by many unfolded proteins (65, 79, 135). In some cases, however, there must be more specialized requirements since some heat-induced chaperones are quite specific in their choice of target proteins. Hsp90 forms stable associations with only certain classes of proteins, and chaperones like hsp47 are specific for individual proteins (e.g. collagen) (181). In the sections below, we focus on the families of heat-inducible chaperones and discuss how they interact with each other and how they function to protect organisms from stress.

Hsp70

THE HSP70 FAMILY   Eukaryotes produce a multitude of proteins in the hsp70 family. These proteins share ~50% amino-acid identity with each other and

with the DnaK protein of *E. coli* (Figure 2). Distinct members are found
in the ER, mitochondria, and chloroplasts. Others are found in the nucleus
and cytoplasm, some constitutively expressed, others induced by heat or
cold; some concentrate in the cytoplasm, others shuttle between the nucle-
olus, the nucleus and the cytosol. Multicellular organisms contain several
tissue-specific versions (e.g. the testis-specific hsp70s) (79, 149). For years
it was thought that the *E. coli* genome encodes only one hsp70 protein,
DnaK, which is constitutively expressed and induced by heat. Recently,
however, two related proteins have been identified. One, Hsc66, shares
40% amino-acid identity with DnaK. It is found in a bicistronic operon
with ferredoxin, suggesting that it may play a specific role in chaperoning
the folding or assembly of this protein (231). The other protein, MreB,
shares 27% identity with the N-terminal domain of hsp70 and is involved
in cell division (95). More distantly related members of the hsp70 family
are being identified in eukaryotes as well (see (66)).

HSP70 FUNCTIONS AT NORMAL TEMPERATURES   Hsp70 proteins bind to small
peptides (with a preference for hydrophobic amino acids (65; M-J Gething,
personal communication), to nascent chains on polysomes (13), to proteins
that have been targeted to the wrong cellular compartment (16), to certain
mutant proteins (78), to certain protein subunits that are expressed in the
absence of their partners (178), and to certain oligomeric proteins in the
process of assembly or disassembly (46, 76, 216). In binding to these
substrates, hsp70 proteins participate in a variety of protein folding, unfold-
ing, assembly, disassembly and translocation processes. For example, one
hsp70 protein catalyzes the disassembly of clathrin cages (46, 216); another
facilitates the assembly of proteins in the endoplasmic reticulum (178);
others facilitate the transport of proteins across membranes, one working
in the cytosol to maintain the protein in a translocation-competent state,
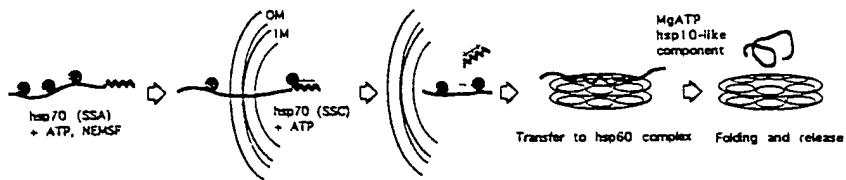
Figure 3   A schematic view of cooperation between hsp70 proteins and hsp60 in the import and
folding of mitochondrial proteins. Two different hsp70 proteins participate in import. One
(exemplified by the yeast Ssa proteins) keeps the substrate in a conformation that is competant
for transport across the membrane; the other (exemplified by the yeast Ssc1 protein) accepts the
unfolded protein inside the matrix and passes it to hsp60 for folding. (Figure courtesy of A.
Horwich.)

and others working in the ER or in the mitochondrial matrix to accept the nascent chain as it traverses the membrane (See Figure 3; 37a, 40, 47, 184, 268a). The favored hypothesis that explains the role of hsp70 in these processes is that hsp70 binds to hydrophobic surfaces, preventing adventitious associations and stabilizing target proteins in a fully or partially unfolded state (198). ATP promotes release of the substrate, allowing it to continue folding, transport, or assembly (79).

While hsp70 proteins are similar in their general function of chaperoning unfolded proteins, specific functions have evolved for individual family members. In some cases, members are dispersed to separate cellular compartments and function uniquely in those locations. Additional specialization exists within particular compartments. For example, in the yeast *S. cerevisiae*, two families of hsp70 proteins are expressed in the nuclear/ cytoplasmic compartment, the Ssa and Ssb proteins (43, 276). *ssa* mutations cause strong defects in the transport of precursors across mitochondrial and ER membranes (47, 184), while mutations in the Ssb proteins do not. Rather, *ssb* mutations result in defects in protein synthesis, consistent with a role for these proteins in chaperoning nascent chains on polyribosomes (183). While the Ssa and Ssb proteins may have overlapping functions, they have clearly developed a considerable degree of specialization.

HSP70 STRUCTURE   All of the hsp70 proteins studied to date fold into two domains. The N-terminal domain is the most conserved (Figure 2) and contains a high affinity ATP-binding site (64, 162). The C-terminal domain is less conserved and is responsible for binding substrate proteins and polypeptides, perhaps in a manner similar to that of the MHC proteins (212a). This organization suggests that hsp70 proteins share a common mechanism for using the energy of ATP while recognizing a variety of substrates. The two domains are interdependent: ATPase activity is strongly stimulated by substrate binding, and the release of substrates is dependent upon the nature of the bound nucleotide. Nucleotide binding, substrate binding, and phosphorylation alter the conformation, oligomeric structure, and ATPase activity of hsp70 proteins ((17, 75, 79, 98, 192, 215, 258a); AL Goldberg, personal communication), but a detailed mechanistic description of how these changes govern hsp70 function is just being formed. It is generally thought that ATP hydrolysis is required for the release of substrates from hsp70 because an analog of ATP that resists hydrolysis, ATP-γ-S, does not promote substrate release. However, closer examination reveals that, after the addition of ATP to hsp70-substrate-ADP complexes, complete substrate dissociation is achieved before stoichiometric ATP hydrolysis can occur. Thus, it now appears that it is the transition of hsp70 from an ADP-bound to an ATP-bound form that drives substrate release (192a).

PARTNER PROTEINS   Other hsps potentiate certain activities of hsp70 proteins. The clearest examples come from in vitro analysis of the *E. coli* DnaK protein and involve the hsps GrpE and DnaJ. In the initiation of bacteriophage λDNA replication, the λP protein tightly binds DNA helicase and recruits it to the replication origin through interaction with the λO protein. The interaction between λP and helicase must be broken for replication to proceed. This is accomplished by DnaK in cooperation with DnaJ and GrpE. DnaJ and GrpE function to enhance interaction between DnaK and the helicase/λP complex and to stimulate the ATPase activity of DnaK by promoting ATP hydrolysis and ADP/ATP exchange (reviewed in (77, 291)). DnaJ and DnaK also cooperate to promote plasmid P1 replication. In this case, activation of the P1 initiator protein, RepA, proceeds in two steps. First, DnaJ binds inactive RepA dimers, targeting them for association with DnaK. DnaK then stimulates the dissociation of RepA dimers to RepA monomers, the active form of the protein (148a, 277, 278). DnaJ, GrpE, and DnaK also cooperate in chaperoning nascent chains on polyribosomes in vitro. DnaJ binds to nascent chains co-translationally and arrests their folding while the addition of DnaK and GrpE reinitiates folding (104).

Finally, DnaJ, GrpE, and DnaK cooperate to regulate the expression of hsps by controlling the level and activity of the heat-shock regulator σ^{32} (28, 41). Mutations in *dnaJ, grpE,* or *dnaK* increase the basal synthesis of hsps and delay feedback repression of hsp synthesis during adaptation to high temperatures. The mutations affect hsp synthesis in three ways: they cause enhanced translation of the σ^{32} message, reduced proteolysis of σ^{32} protein, and increased transcriptional activity of σ^{32} protein (30, 248, 257). In vitro, DnaJ and DnaK bind σ^{32} directly and, together with GrpE, specifically suppress σ^{32}-directed transcription (72, 145). It is not yet clear how these hsps affect σ^{32} degradation and translation. Given their capacity to interact directly with σ^{32}, they may enhance presentation of σ^{32} to the degradative machinery and interfere with translation of the σ^{32} message by interacting with σ^{32} nascent chains. Because DnaJ and DnaK also bind denatured proteins, an increase in denatured substrates after shift to high temperatures may recruit the chaperones away from σ^{32}, thereby inducing hsp synthesis. As the hsps accumulate, rebinding to σ^{32} would provide an elegant mechanism for feedback regulation (28, 41, 248).

It is likely that eukaryotic hsp70 proteins function with DnaJ and GrpE homologs. Eukaryotic cells contain at least one GrpE homolog (K Palter, personal communication) and a whole family of DnaJ-related proteins targeted to various cellular locations (21, 32a). Deletions of the yeast *DnaJ* homolog, *YDJ1*, retard growth at 30°C and prevent growth at 37°C. Expression of the *E. coli* DnaJ protein suppresses the growth defect of the *ydj1* cells at 30°C. Moreover, purified Ydj1 protein stimulates ATP hydrolysis by hsp70 (32).

HSP70 FUNCTIONS IN STRESS TOLERANCE IN VITRO   In only two cases have stress-specific functions of hsp70 been studied in detail in vitro. The pioneering study examined the effect of DnaK on the denaturation and aggregation of RNA polymerase (RNAP) at 42°C (237). DnaK is able both to protect RNAP from inactivation by high temperature and to reactivate previously heat-dena- tured RNAP, but the two processes appear to be mechanistically distinct. For protection, ATP is not required, and a mutant protein encoded by the DnaK[756] allele is functional. For reactivation, ATP is required and DnaK[756] protein is not functional. In these experiments, a remarkable 60-fold molar excess of DnaK to RNAP was required for reactivation. Recently, DnaJ and GrpE have been shown to improve the efficiency of DnaK in this reaction (C Georgopoulos, personal communication).

The effects of hsp70 on the denaturation and refolding of firefly luciferase in vivo and in vitro currently provide the most detailed picture of hsp function in stress tolerance (228). Luciferase expressed in *E. coli* is rapidly inactivated when cells are shifted to 42°C, but is reactivated when cells are returned to 30°C. Inactivation occurs in either the presence or absence of DnaK, DnaJ, or GrpE, but all three proteins are required for reactivation. In vitro, reactivation of heat-denatured luciferase requires the presence of DnaJ during the initial heat-denaturation step. The protein suppresses ag- gregation of luciferase by forming a complex. Once DnaJ has bound to the denatured substrate, DnaK and GrpE promote the release of refolded lucif- erase by a mechanism that is dependent upon ATP. Luciferase refolding is achieved with high efficiency at nearly stoichiometric concentrations of DnaK, DnaJ, and GrpE. In contrast to the RNA polymerase studies (described above), DnaK alone is completely ineffective in reactivation, even when present at a 100-fold molar excess.

HSP70 FUNCTIONS IN STRESS TOLERANCE IN VIVO   In most organisms, hsp70 proteins are among the most prominent proteins induced by heat (Figure 1), and early experiments showed a close correlation between the induction of these proteins and the induction of tolerance to high temperatures (142, 144, 250). When the role of hsp70 in chaperoning unfolded proteins at normal temperatures first became apparent, a role in chaperoning proteins damaged by stress seemed a natural and logical extension (198, 215). It is now clear that the heat-inducible forms of hsp70 do play a central role in stress tolerance, by promoting growth at moderately high temperatures and/or protecting organisms from killing at extreme temperatures. The importance of hsp70 in stress tolerance, however, varies to a surprising degree in different organisms.

E. coli   Deletion of the *E. coli dnaK* gene is not lethal, although it severely reduces the cellular growth rate at 30°C and eliminates growth both at high

(40°C) and at low (11–16°C) temperatures (29, 160a). Consistent with the role of DnaK in hsp regulation, $dnaK^-$ strains strongly over-express other hsps. Most suppressors of the 30°C growth defect map to σ^{32} and reduce hsp over-expression, suggesting that the major function of DnaK at 30°C is to downregulate the expression of other hsps (30). These suppressors do not, however, potentiate growth at higher temperatures, where the general chaperone functions of DnaK presumably assume greater importance. Surprisingly, $dnaK$ mutations have only a very modest effect on induced tolerance to extreme temperatures. Mild pre-treatments (42°C for 5 min) dramatically increase survival at 50°C, but they do so to nearly the same extent in wild-type cells and in $dnaK$ mutants (B Bukau, personal communication). Instead, two mechanisms appear to provide tolerance to extreme temperatures in *E. coli*, one involving proteins under control of the stationary phase regulator, σ^s, and the other involving other hsps (B Bukau, personal communication; 106, 242, 287). These mechanisms are only beginning to be characterized.

S. cerevisiae   As discussed above, yeast cells express three heat-inducible nuclear/cytosolic hsp70 proteins, which form an essential complementation group with a constitutive hsp70 protein. Eliminating all three heat-inducible genes prevents growth at moderately high temperatures (37°C), but does not cause any defect in survival at extreme temperatures (50°C), either with or without a tolerance-inducing 37°C pretreatment (276). These mutations reduce survival at high temperatures only when they are combined with mutations in the yeast *HSP104* gene, which encodes the major protein responsible for thermotolerance in yeast (219; see below). Thus, as seems to be the case in *E. coli*, the heat-inducible hsp70 proteins of *S. cerevisiae* are primarily responsible for helping cells cope with the stress of growth at moderately high temperatures, rather than for helping them survive extremes.

D. melanogaster   In contrast to *E. coli* and *S. cerevisiae*, hsp70 appears to be the major protein involved in tolerance to extreme temperatures (40–42°C) in Drosophila. Here, hsp70 is amplified in the genome and regulated by temperature to an unusual degree. As discussed earlier, hsp70 is virtually undetectable at normal temperatures (25°C) and is induced 1000-fold upon heat shock (37°C) (265). Cell lines transformed with extra copies of the wild-type *hsp70* gene accumulate hsp70 at a faster rate than wild-type cells when shifted to 37°C and acquire tolerance to more extreme temperatures at a faster rate. Cells transformed with hsp70 antisense genes accumulate hsp70 at a slower rate and also acquire tolerance more slowly (240). The antisense gene-containing cells also overexpress all other hsps

at 37°C, confirming the importance of hsp70 in regulating the heat-shock response (41, 49), and suggesting that other hsps cannot compensate for hsp70 in providing thermotolerance. When hsp70 was expressed from a heterologous promoter at 25°C, without the induction of other hsps, it was sufficient to provide tolerance to direct exposure to 42°C. This result indicates that hsp70 induction alone is sufficient for thermotolerance in Drosophila cells (240). More recently, these observations were extended to the organismal level using fly strains that carry twelve extra copies of the wild-type *HSP70* gene (274). The acquisition of thermotolerance in early embryos of these strains parallels the increase in hsp70 expression, demonstrating that the accumulation of hsp70 is a rate-limiting step in the acquisition of thermotolerance (274). At this stage in development, the embryo is a complex assemblage of more than 10,000 cells. The significant increase in thermotolerance resulting from overexpression of hsp70 suggests that it may be possible to alter the intrinsic thermal resistance of complex multicellular organisms by deliberate genetic intervention. Surprisingly, however, the transgenes do not increase survival in older embryos. This might be due to a failure in expression of the transgene in some critical tissue or to the fact that other tolerance factors become rate-limiting at later stages (274).

A novel mechanism for controlling the activity of hsp70 was discovered in these thermotolerance experiments. Although tissue culture cells expressing hsp70 from a heterologous promoter displayed high levels of heat tolerance, they stopped growing (59). After several days of continuous induction, growth resumed, accompanied by the sequestration of hsp70 into large granules. Similar granules appear in wild-type cells ~12 hrs after a standard heat shock, and this sequestration parallels the loss of thermotolerance. Granules also appear in wild-type six-hour embryos within a few minutes of recovery from mild heat-treatments, and this sequestration of hsp70 correlates with a remarkably rapid loss of thermotolerance (M Welte, J Feder & S Lindquist, unpublished observations). Apparently, although the heat-inducible form of hsp70 is beneficial for survival during exposure to high temperatures, it is detrimental for growth and cell division (59). In early embryos, which have rapid rates of cell division, the rapid inactivation of hsp70 by sequestration into granules may be essential for the resumption of normal development after heat shock. Toxic effects of hsp70 on growth may also explain two puzzling observations. First, nurse cells provide the oocyte with hsp90 and small hsps but not with hsp70 (289). Second, in very early embryos (0–3 hr), which have the highest known rates of nuclear division of any eukaryote, hsp70 cannot be induced by heat shock (53, 93). Thus, there is a complete absence of hsp70 in 0–3 hr embryos. During this period, embryos are extremely sensitive to heat, suggesting that the detri-

mental effects of hsp70 on cell growth outweigh the risk of death from the toxic effects of heat. Thus, engineering heat tolerance during particular stages of development will require a deeper understanding of the effects of hsps on normal cells.

Vertebrates   The role of hsp70 in induced thermotolerance has been studied extensively in vertebrate cells (97, 191). Some data must be cautiously interpreted since constitutive expression of hsp70 was altered in many experiments. Given the role of hsp70 in regulating other hsps, a difference in constitutive hsp70 expression might have pleiotropic effects. Moreover, a few reports contradict the general trend and suggest that hsp70 is not required for thermotolerance (reviewed in (33)). However, the many different approaches that indicate a role for hsp70 in thermotolerance provide a compelling argument. Contradictory reports are likely due to the existence of additional tolerance mechanisms in some cells.

Support for the role of hsp70 in thermotolerance in vertebrates includes: (a) In several different cell types exposed to a wide variety of tolerance-inducing treatments, closer correlation exists between the induction of thermotolerance and the accumulation of hsp70 than exists for the accumulation of any other hsp (142, 144); (b) The selection of heat-resistant cell lines by hyperthermic treatments yields cells that over-express hsp70 proteins (4, 138); (c) Microinjection of antibodies against hsp70 prevents survival of fibroblasts at 45°C, while microinjection of control antibodies has no such effect (212); (d) Microinjection of mouse oocytes with hsp70 mRNA increases their capacity to survive short heat shocks (103a); (e) Transformation of rat cells (143) and monkey cells (5) with constitutively expressed human hsp70 genes dramatically increases thermotolerance.

In the last case (143), a careful two-dimensional analysis of total cellular proteins demonstrates that increased basal expression of hsp70 is the only substantial alteration in the protein profile, making this study the most convincing demonstration of the importance of hsp70 in mammalian thermotolerance. In a surprising extension of these studies, deletion of most of the highly conserved N-terminus of hsp70 did not impair its capacity to provide thermotolerance, suggesting that this domain is not required for thermotolerance. However, the effects of the mutant protein on the expression of other proteins at normal temperatures were not examined. Because similar mutations in the Drosophila hsp70 protein upregulate the expression of hsps (240), the induction of endogenous hsps by the exogenous mutant hsp70 may account for thermotolerance in these cells.

While we have learned a great deal about the general biochemical activities of hsp70 and its importance in protecting organisms at high temperatures, we still have not defined the critical biological processes that are protected

by it at high temperatures. By immunofluorescent localization, eukaryotic hsp70 concentrates at membranes, in nuclei, and in nucleoli (198a, 264a, 273a). This pattern is consistent with other observations that suggest that hsp70 is particularly important in these locations: (a) Members of the yeast hsp70 family that are both constitutive and heat-inducible promote the translocation of proteins across mitochondrial and ER membranes (37a, 47, 184, 268a); (b) Purified hsp70 repairs heat-induced damage to some nuclear functions (e.g. mRNA splicing) in yeast cell-free systems (J Vogel, S Lindquist, unpublished); (c) Constitutive expression of Drosophila hsp70 accelerates the recovery of nucleolar morphology after heat shock (137a, 197a). Because heat shock has a plethora of deleterious effects on cellular morphology (188), it is unclear which of the many targets of hsp70 action are the most critical for survival at high temperatures.

Hsp60 (GroEL, Chaperonin-60)

HSP60 CONSERVATION AND STRUCTURE   Hsp60 proteins are found in the cytosol of bacteria (where they are known as GroEL proteins), in the matrix compartment of mitochondria, and in the stromal compartment of chloroplasts (where they are known as chaperonin-60 proteins) (56, 98, 155). GroEL is one of the most abundant proteins in the cell at normal temperatures, accounting for 1% to 2% of total protein, while hsp60 accounts for ~1% of mitochondrial matrix protein. All of these proteins share nearly 60% amino-acid identity along the entire length of the protein (103, 208; Figure 2). Most share a common oligomeric structure, a "double doughnut" of two seven-membered rings (56, 105, 165), while the mitochondrial protein forms a single seven-membered ring (267a). Hsp60 has an ATPase activity that increases with temperature, and the binding of ATP induces a major conformational change in the structure of the oligomer (217, 260).

Hsp60 functions are strongly dependent upon another heat-inducible protein, hsp10 (also known as GroES or chaperonin-10). Like the hsp60 proteins, hsp10 proteins are found in bacteria, mitochondria, and chloroplasts (56, 98). Moreover, the hsp10 proteins also form homo-oligomeric ring-shaped particles, in this case, single, seven-membered rings (34). The hsp10 particle binds to one end of the hsp60 particle and regulates its ATPase activity and substrate associations (137, 217).

HSP60 FUNCTIONS AT NORMAL TEMPERATURES   Hsp60 binds unfolded proteins and promotes their folding. This function has been analyzed with a variety of substrates in vitro, and several common features can be distilled. Hsp60 shows little or no affinity for native proteins, but when urea- or guanidine-denatured substrates are diluted into physiological buffers, it

captures them with high efficiency. While bound to hsp60, the denatured substrates acquire elements of secondary structure (134, 157), but the development of tertiary structure is inhibited. Thus, proteins associated with hsp60 are highly sensitive to proteolytic digestion and expose hydrophobic residues that are buried in the native structure (157). In most cases, hsp10 (GroES) and ATP are required for further folding of the substrate protein and release from hsp60 (85, 131, 157, 267). In other cases, however, the addition of nonhydrolyzable ATP analogs is sufficient (267). It is unclear why some substrates require hsp10 and hydrolyzable ATP, whereas others do not.

Hsp60 is sometimes described as a catalyst of protein folding, but this description misrepresents its function. One hsp60 particle will promote the folding of many substrate molecules, fulfilling the multiple turnover requirement of a catalyst (157). However, while hsp60 enhances the yield of folded protein, it does not generally enhance the rate of folding. In fact, in most cases, hsp60 markedly reduces the rate of folding. For folding in vivo, reductions in the rate of folding due to transient association with hsp60 should be insignificant, given the length of time required for translation in the first place. The increased yield of folded protein is of far greater importance in the highly concentrated environment of the cell (56, 98, 155). In keeping with their central roles in protein folding, both the bacterial and the mitochondrial hsp60s are essential for growth at all temperatures (36, 58, 208), as is the bacterial hsp10 (58).

Mitochondria provide a particularly valuable system for studying the physiological functions of hsp60 because the posttranslational import of proteins into mitochondria naturally separates synthesis from folding. In yeast, temperature-sensitive (ts) mutations in hsp60 result in the misfolding and aggregation of a wide variety of oligomeric matrix proteins, including F_1-ATPase and ornithine transcarbamlyase, at the nonpermissive temperature (36, 111). The role of hsp60 in the assembly of oligomeric proteins appears to be in the production of assembly-competant monomers (reviewed in (98, 155)).

HSP60 FUNCTIONS IN STRESS TOLERANCE   The importance of hsp60 for protein folding in vitro is strongly temperature-dependent. When urea- or guanidine-denatured Rubisco is diluted into physiological buffer, the protein aggregates, and, unless hsp60 is present, little or no active enzyme is formed at temperatures above 25°C. At lower temperatures, however, the recovery of active enzyme increases. Below 10°C complete activity is recovered in the absence of hsp60 (85, 268). Similarly, urea-denatured rhodanase is dependent upon hsp60 and hsp10 for refolding at 37°C at high concentrations, but virtually independent of the chaperones at lower concentrations and temperatures (167, 168).

In yeast mitochondria, hsp60 associates with a wide variety of proteins at high temperatures. This association presumably prevents protein aggregation and promotes refolding when cells are returned to lower temperatures. Indeed, when dihydrofolate reductase (DHFR) is provided with a signal sequence for mitochondrial import and expressed at normal temperatures, hsp60 is required to prevent inactivation of previously synthesized enzyme when cells are shifted to high temperatures (156). Hsp60 also prevents the aggregation of proteins that denature at physiologically relevant temperatures in vitro. DHFR and α-glucosidase denature between 40–45°C and form irreversible aggregates. When hsp60 is present during heating, however, aggregation of these proteins is suppressed through the formation of binary complexes. When the temperature is reduced, active enzyme can be recovered upon addition of hsp10 and ATP (110, 156).

Overexpression of hsp60 (GroEL) in *E. coli* suppresses a variety of conventional, temperature-sensitive (ts) mutations (264), probably by preventing the aggregation of mutant proteins that unfold at high temperature and by facilitating their refolding. Less conventional, temperature-sensitive-folding (tsf) mutations in two different bacteriophage P22 proteins behave differently with respect to rescue by hsp60. When expressed and allowed to fold at low temperatures, several mutant P22 tailspike proteins are stable at high temperatures (melting temperatures > 82°C). However, these same proteins misfold and form inclusion bodies when expressed in vivo at 37–42°C. Their temperature-sensitive phenotypes are due to the increased tendency of the mutant proteins to go off-pathway during synthesis or folding (286). GroEL is capable of interacting with these mutant tailspike proteins, but it does not improve the yield of properly folded protein in vitro and provides only a minor increase in yield in vivo (26; S Sather & J King, personal communication). On the other hand, a large number of tsf mutations in the P22 coat protein are rescued by GroEL. The plating efficiency of phage carrying these mutations is increased by five orders of magnitude at 40°C in hosts that overexpress both GroEL and GroES (87; J King, personal communication.) Tsf mutations are uncommon, perhaps because ts mutations in structural oligomeric proteins have been little studied. In any case, determining why one class of tsf mutations is affected by GroEL, while another is not, will likely provide important insights into the specificity of hsp60 functions in vivo.

The GroEL protein is strongly induced by heat, increasing to a remarkable 10–15% of total cellular protein (182). (The organellar proteins are also induced by heat but to a lesser degree, increasing two- to threefold (164)). Consistent with the central role of GroEL in protein folding, this increase in GroEL concentration is essential for growth at high temperatures. An *E. coli* strain carrying a deletion in the σ^{32} gene cannot induce hsps or grow above 20°C (130). Second-site suppressors of this defect map primarily to

the *groEL* (hsp60) promoter. Over-expression of GroEL alone permits growth at temperatures up to 40°C in the σ^{32} mutant. (For growth to 42°C, however, over-expression of DnaK is also required.) Whether hsp60 plays a significant role in protecting cells from short exposures to more extreme temperatures is unknown. However, in the many cases tested, GroEL is unable to promote renaturation and refolding of previously aggregated proteins.

COOPERATION BETWEEN DNAJ, HSP70 AND HSP60   A major revelation of recent work is that DnaJ, hsp70, and hsp60 can work together to provide a pathway for protein folding. Both hsp70 and hsp60 are required in mitochondria for the translocation of proteins into the organelle and for their assembly into functional complexes (see Figure 3). Hsp70 keeps the proteins unfolded as they enter the organelle, maintaining them in a state that is competent for transfer to hsp60 (184). In turn, hsp60 promotes the folding and assembly of these proteins into oligomeric structures (98).

In vitro studies with urea-denatured rhodanese also point to an ordered series of interactions with substrates during folding. First, DnaJ binds the unfolded protein and stabilizes its interaction with DnaK. GrpE then couples the ATP hydrolysis by DnaK to transfer of the substrate to GroEL and GroES, which, in turn, promote productive folding and release of native substrate (56, 136).

TF55/TRiC

THE TF55 PROTEIN   The central role that hsp60 proteins play in protein folding and stress tolerance in bacteria and in eukaryotic organelles begs the question: what protein(s) provide these functions in the cytosolic and ER compartments of eukaryotes? One answer has come from an unexpected source. In the thermophilic archaebacteria *Sulfolobus shibatae*, high temperatures increase the expression of a single 55-kd protein, TF55 (261). The oligomeric structure of TF55 is similar to that of hsp60, consisting of two stacked nine-membered rings. Other species of archaebacteria contain similar particles that are abundant at normal temperatures and strongly induced by heat (e.g. a shift from 100°C to 108°C). For example, particles purified from *Pyrodictium occultum* consist of stacked eight-membered rings containing two polypeptides of 56 and 59 kd in a 1:1 ratio. Detailed structural analysis of this particle by conical tilt electron microscopic reconstruction reveals striking similarity to hsp60 on both its front and side faces, although the central cavity of the archaebacterial particle is larger and could presumably accommodate larger polypeptides (73, 202, 217, 260). Both of the archeabacterial particles have intrinsic ATPase activities comparable to that of hsp60, and like hsp60, these activities increase with temperature over the physiological range where they function (202, 260).

The TF55 particle shares at least one other important characteristic with the hsp60 particle, an ability to bind to a wide variety of denatured proteins (260; J Trent. personal communication). However, unlike hsp60, TF55 has not yet been shown to facilitate the folding of any denatured substrate, perhaps because an essential cofactor analogous to hsp10 has yet to be identified. As expected for a general chaperone, the TF55 protein is very abundant at normal temperatures and is envisioned to play a central role in normal protein folding. Furthermore, TF55 is almost certainly the central protein involved in thermotolerance in *Sulfolobus*. Unlike eubacteria and eukaryotes, which strongly induce 5–15 proteins in response to high temperatures, *Sulfolobus* produces only TF55. The induction of this protein is closely correlated with the induction of tolerance to otherwise lethal temperatures (261). Unfortunately, methods for creating site-directed mutations are not yet available in *Sulfolobus*, preventing a direct genetic test of this hypothesis.

TRiC   A possible candidate for a cytosolic hsp60-like chaperone was revealed in a search of the Genbank database with the TF55 sequence. A high level of homology (40% amino-acid identity) was observed between TF55 and a group of cytosolic proteins previously identified in yeast, Drosophila, and mammals. These proteins are known as the T-complex proteins, or TCPs, because the mammalian proteins are encoded within the murine T-complex. The TCP proteins also form double ring-shaped particles (TRiC = TCP containing ring complex), whose front and side views resemble those of hsp60. The composition of these particles is more heterogeneous, however, consisting of at least several different proteins in the 52–65 kd range (71, 141, 177a). Notably, all of these proteins show a low, but convincing, level of amino-acid sequence homology with the hsp60 family of proteins (90, 141).

Disruption of the *TCP1* gene in yeast is lethal. A cold-sensitive mutant produces anucleate and multinucleate cells at the nonpermissive temperature and displays aberrant microtubule staining (263). Temperature-sensitive alleles cause arrest at the G2-M stage of the cell cycle and are defective in spindle biogenesis (170). These phenotypes suggest a role for TCP in microtubule metabolism. Indeed, TRiC chaperones the folding of tubulin in vitro, in a manner that closely resembles that of hsp60 (282). Purified TRiC can also chaperone the folding of purified, denatured tubulin in vitro (71). The release of folded tubulin from purified TCP requires additional protein factors (74). These factors have not yet been characterized, but it is tempting to speculate that they function with TRiC in a manner analogous to the way in which hsp10 functions with hsp60.

Whether TRiC plays a general role in chaperoning protein folding in the eukaryotic cytosol of a magnitude comparable to that of hsp60 in bacteria

and organelles is still a matter of controversy. TRiC does promote the folding of actin (73), denatured luciferase (71), and the phytochrome photoreceptor (177a) in vitro in an ATP-dependent manner. However, one laboratory that has successfully employed TRiC to fold tubulin and actin reports that TRiC is unable to facilitate the folding of several other denatured test substrates, including cyclin B, H-ras, cap-binding protein, and α and β globin. In fact, TRiC does not even form a binary complex with these denatured substrates (74). Another argument against a central role for TRiC in general protein folding is its low abundance in most cell types. However, recent improvements in the purification of TRiC suggest it is of greater abundance than initially supposed (A Horwich, personal communication; see also 177a). Finally, while GroEL and TF55 are strongly induced by heat, TRiC proteins are not. There is as yet no suggestion that the TRiC plays a role in eukaryotic stress tolerance.

Hsp90

THE HSP90 FAMILY   Members of the hsp90 family are present in the cytosolic/nuclear compartment of all eukaryotes examined and are also found in the ER of higher eukaryotes. Although no organellar species have yet been found, an *E. coli* homolog, HtpG, shares ~40% amino-acid identity with the eukaryotic proteins (Figure 2). HtpG is a moderately abundant protein at normal temperatures and is strongly induced by heat, yet deletion of the gene has no effect on growth at normal temperatures and produces only a very subtle reduction in growth at high temperatures (10). In the yeast *S. cerevisiae*, on the other hand, hsp90 proteins are essential at all temperatures, indicating that the eukaryotic proteins, which differ from the prokaryotic protein not only by general sequence divergence but also by large sequence insertions, have acquired novel, vital functions (22).

Hsp90 proteins from a variety of sources reportedly bind ATP (44) and have peptide-stimulated ATPase activities (179). A puzzling aspect of this work is that hsp90 proteins purified from different sources have very different levels of ATPase activity. Moreover, although hsp90 proteins contain a sequence that weakly matches an ATP-binding consensus sequence (44), site-directed mutagenesis of this region has no detectable effect on hsp90 functions in vivo (S Bohen & K Yamamoto, personal communication). The fact that hsp90 proteins copurify with kinases, in some cases through many steps of purification (175), makes a more detailed analysis of hsp90 ATP-binding functions imperative.

HSP90 FUNCTIONS AT NORMAL TEMPERATURES   Members of the hsp90 family interact with many other cellular proteins, including casein kinase II (175),

the heme-regulated eIF-2α kinase (213), several steroid hormone receptors (206), oncogenic tyrosine kinases (25), calmodulin (172), actin (174, 187), and tubulin (67, 210). In vitro, hsp90 functions as a general chaperone, although its effects are extremely modest compared to those of hsp60. When denatured citrate synthase is diluted into physiological buffer, hsp90 increases the yield of active enzyme threefold and, in similar experiments, enhances the restoration of binding activity in antibody Fab fragments 1.5-fold (279). It is not clear whether these modest effects are due to a requirement for other proteins (i.e. hsp70 and peptidyl prolyl isomerases) or to an inherent specificity in hsp90 that normally restricts its functions to certain types of target proteins. (Both issues are addressed below.) The in vitro analysis of casein kinase II, a protein for which there is some evidence for an interaction with hsp90 in vivo, provides stronger evidence for a chaperone function of hsp90 (175). In this case, hsp90 prevents aggregation of the kinase, promotes disaggregation of previously aggregated kinase, and enhances the activity of the kinase more than twentyfold.

Although hsp90 interacts with a wide variety of proteins, it displays a high level of discrimination in these associations. For example, it forms stable complexes with several steroid receptors, including the estrogen, progesterone, androgen, glucocorticoid, and dioxin receptors of vertebrate cells (206, 207, 239), and the antheridiol receptor of the fungus *Achlya ambisexualis* (27), but not with other members of the steroid receptor superfamily, such as the retinoic acid receptors and the thyroid hormone receptors (206, 239). Similarly, it interacts strongly with several different oncogenic members of the src kinase family, but interacts weakly, if at all, with their cellular homologs (25). The nature of this discrimination is unclear.

In vitro and in vivo analyses of the interaction between hsp90 and these two very different types of target proteins, steroid receptors, and *src* kinases, offer complementary views of hsp90 function. In vitro, steroid receptors that complex with hsp90 are unable to bind DNA unless this association is disrupted either by hormone or by a change in pH, salt, temperature, or metal ion chelation (206, 207). Similarly, newly synthesized *src* kinases are inactive while associated with hsp90 and are only activated when they insert into the membrane and lose this association (25). While these experiments suggest that hsp90 represses both proteins, genetic analyses suggest that it activates them. Mutations that reduce the level of hsp90 reduce both the level of hormone-activated, receptor-mediated transcription (203) and of *src*-mediated tyrosine phosphorylation (281). The picture that emerges from these seemingly disparate results is that hsp90 helps the steroid receptors and the oncogenic tyrosine kinases assume an activation-competent conformation, but they remain inactive as long as hsp90 stays associated

with them. Thus, two characteristics appear to distinguish hsp90 from the other abundant hsp chaperones, hsp70 and hsp60. First, hsp90 displays a high specificity in associating with particular target proteins. Second, the interactions between hsp90 and at least some target proteins are long-lived and have acquired important regulatory features.

A detailed, mechanistic description of hsp90 functions with steroid receptors and oncogenic kinases is not yet possible, primarily because reconstitution of hsp90/substrate complexes from purified components has not yet been achieved. Salt-disrupted complexes have been reconstituted in reticulocyte lysates, however (112, 225, 238), with results that suggest that association of hsp90 and its target proteins requires hsp70 and ATP. Gentle fractionation methods have also helped to identify additional components of hsp90/substrate complexes (179, 238, 254). Some of these proteins are of unknown function, but others have been identified as prolyl isomerases (described below), raising the intriguing possibility that this group of proteins collaborates with hsp70 and hsp90 to promote protein folding.

HSP90 FUNCTIONS IN STRESS TOLERANCE   Genetic analysis in *S. cerevisiae* demonstrates that the quantity of hsp90 required for growth increases as the temperature increases, such that 20- to 30-fold more protein is required for growth at 39°C than at 25°C (22; L Arwood & S Lindquist, unpublished results). While hsp90 plays a key role in helping cells cope with the stress of growth at moderately high temperatures, its importance in helping cells survive extreme temperatures is less clear. Hsp90 is the only protein whose expression is noticeably increased in a particular temperature-resistant Chinese hamster ovary line (283). Also, mammalian cells with reduced hsp90 concentrations (due to antisense RNA expression) are killed somewhat more rapidly than wild-type cells at extreme temperatures (9). In yeast, however, mutational analysis in two different laboratories indicates that the protein is not required for tolerance to extreme temperatures (22, 124).

The Small Hsps

CONSERVATION AND STRUCTURE   Of the major hsp families, the small hsps (smhsps) are the least conserved (Figure 2) and the most elusive in function. The proteins vary greatly in size (from 12 kd to 40 kd) and amino-acid composition. Nevertheless, they constitute a distinct family that includes the α-crystallin proteins of the vertebrate eye, and can be recognized by (a) limited amino-acid identity (~15–20%), (b) similar hydropathy profiles, (c) a conserved hydrophobic sequence element in the C-terminal region, (d) a distinct oligomeric structure that sediments on sucrose gradients at 15–20S, and (e) induction by heat and a variety of developmental signals (6, 149). The smhsps are found in prokaryotes (mycobacteria) and in the eukaryotic

cytosol. They are particularly abundant in plants, which also encode smhsps that are targeted to the endomembrane system and to chloroplasts (for review, see (266)). The conservation of these proteins across prokaryotic and eukaryotic kingdoms and in different eukaryotic compartments strongly suggests an important cellular function.

The cytosolic smhsp proteins show a complex and baffling pattern of relocalization in response to heat and a variety of other stimuli (6, 188, 214). In both plants and mammals, under conditions where the proteins are particularly abundant, the 15–20S particles coalesce to form much larger structures (called heat-shock granules), in response to extreme stress. It is not clear whether granules are the functional form of the protein or are merely aberrant aggregates (6, 188). Reports of associations of smhsps with cellular RNAs, photosystem ll components, and other cellular factors have been the subjects of similar controversy (149, 188, 266). The smhsps are phosphorylated in response to heat and a wide variety of other stimuli, and it has been suggested that they function in signal transduction pathways (132, 166) although the evidence to support this role is still very preliminary.

SMHSP FUNCTIONS AT NORMAL TEMPERATURES   One of the most interesting recent developments in the biochemical analysis of the smhsps is that they display elements of chaperone function in vitro. In one study, mouse hsp25 completely suppressed the heat-induced aggregation of native βL-crystallin at 58°C and of native α-glucosidase at 49°C, at a molar ratio of 1:20 and 1:10 (hsp:substrate) (169). In another study, mouse hsp25, human hsp27, and α-crystallin all prevented the heat-induced aggregation of citrate synthase and α-glucosidase at stoichiometric concentrations and promoted the renaturation of these proteins after denaturation in urea (113). In neither study, however, was the formation of a stable binary complex between the smhsps and the substrate proteins reported. Moreover, in contrast with hsp70 and hsp60 chaperoning activities, the chaperoning activities of the smhsps were independent of ATP. The smhsps of avian cells also have dramatic effects on actin polymerization, reducing the low-shear viscosity of F-actin solutions, raising the critical concentration for polymerization in vitro, and disassembling previously assembled F-actin (173).

SMHSP FUNCTIONS IN STRESS TOLERANCE   Genetic analysis of smhsp function gives very different results in mammals and yeast. Hyperthermic selection of mutagenized Chinese hamster ovary cells produced a family of thermotolerant cell lines in which the only detectable change in protein synthesis was the overproduction of hsp27 (38). More convincingly, transfection of naive CHO cells with constitutive hsp25 genes conferred constitutive thermotolerance, and transfection with *metallothionein*-regulated hsp27 genes

conferred metal-regulated thermotolerance (133, 139). Similarly, overexpression of αβ-crystallin in mouse NIH 3T3 cells rendered these cells thermoresistant (5a). To investigate the nature of this protection, several toxic effects of heat shock were examined in thermoresistant CHO cells. Heat treatments disrupted protein synthesis, RNA synthesis, rRNA processing, and protein degradation to a similar extent in thermoresistant hsp27 transformants and wild-type cells (132). However, in the transformants the microfilament (actin) network was dramatically protected from disruption by heat shock (139). It is not clear how microfilament protection in vivo relates to the effects of smhsps on actin polymerization in vitro, but the fact that two very different approaches point to the same potential substrate suggests a role for smhsps in microfilament dynamics.

Yeast cells contain only one major smhsp, Hsp26 (200, 251, 262). Overexpression of this protein provides, at best, only an extremely subtle increase in thermotolerance (251, 262). More importantly, deletions of this gene have no effect on (a) growth at any temperature on a variety of carbon sources, under aerobic or anaerobic conditions, (b) tolerance to extreme temperatures or ethanol in logarithmic and stationary-phase cells, and (c) spore morphogenesis, viability, germination, or thermotolerance (200; J Taulien, M Schlesinger, & S Lindquist, unpublished). Since the yeast hsp100 protein plays such a dominant role in thermotolerance (below), deletions of the *HSP26* gene were introduced into an *hsp104* mutant background. They had no effect on basal or induced thermotolerance (219). Only when cells carry multiple mutations in the *HSP104* gene and in all three heat-inducible hsp70 genes (*SSA1, SSA3*, and *SSA4*) does deletion of *HSP26* reduce survival at high temperatures. Even here, tolerance is reduced only a few fold (J Taulien & S Lindquist, unpublished). Thus, while the smhsps may play an important role in thermotolerance in mammalian cells, they play only a minor role in yeast.

HSPS WITH UNKNOWN TOLERANCE FUNCTIONS

Peptidyl Prolyl Cis-trans Isomerases (PPIs)

Several peptidyl prolyl isomerases (PPIs) have recently been identified as hsps in widely divergent species. PPIs are distinguished from other hsps in that they act as true catalysts of protein folding. In native proteins most peptide bonds exist in the *trans* configuration. Xaa-Proline bonds are a frequent exception because the unique structure of proline relieves normal steric constraints and permits isomerization. The *trans* configuration is produced during protein synthesis, but, in proteins with known structure, ~ 60% have at least one prolyl bond in the *cis* configuration (245). Establishing the *cis* configuration of specific prolyl bonds is a rate-limiting step in folding for many proteins in vitro (226). While isomerization is

constrained by structure in folded proteins, as long as proteins remain unfolded, prolyl bonds will isomerize at 25°C with rate constants of a few minutes. If, as seems likely, spontaneous isomerization can occur while proteins are bound to chaperones, catalysis of isomerization from the *cis* configuration back to the *trans* configuration may be equally important in protein folding in vivo.

Two structurally unrelated families of proteins, the cyclophilins and the FK506 Binding Proteins (FKBPs), catalyze Xaa-Pro isomerization (reviewed in (101, 227)). The two families are named for the immunosuppressive drugs that inhibit their activities, cyclosporin A (CsA) and FK506, respectively. These PPIs are abundant, ubiquitous, and conserved in bacteria and eukaryotes. Moreover, in eukaryotes, members of both families are found in a variety of cellular compartments, including the cytosol, the ER, and mitochondria. Their distribution and conservation suggest that catalysis of prolyl isomerization is of vital importance in vivo. Biochemical and genetic support of this hypothesis, however, is still limited.

The immunosuppressive activities of CsA and FK506 initially suggested that Xaa-Pro isomerization plays a crucial role in the activation of T cells and structural studies suggested that both drugs inhibit PPI activity by mimicking a transition state in prolyl isomerization. However, immunosuppression is achieved at 1/10th the drug concentration required to inhibit PPI activity in the cell (14). Moreover, certain drug analogs that inhibit PPI activity are not immunosuppressive (15, 236). According to current models, PPI inhibition is irrelevant to immunosuppression. Rather, a composite surface created by the drug-enzyme complex provides a gain of function, a high-affinity binding site for the protein kinase calcineurin. It is the binding of calcineurin to this complex that is responsible for immunosuppression (101, 227).

The best evidence for the general physiological importance of prolyl isomerization comes from studies of proteins matured in the ER. In cultured fibroblasts, the addition of CsA inhibits the assembly of collagen triple helices (244). Collagens are rich in hydroxy-proline and it is envisioned that spontaneous isomerizations occurring during the relatively long period these proteins spend bound to chaperones in the ER necessitate re-isomerization prior to assembly. CsA also inhibits the folding of transferrin, perhaps for the same reason (154). In Drosophila, the ninaA protein is a tissue-specific intermembrane cyclophilin localized in the ER of photoreceptor cells. Mutations in *ninaA* cause defects in the production of Rh1 and Rh2, rhodopsins that share several conserved proline residues, but not in Rh3, which does not share these residues (243).

Surprisingly, yeast cells carrying multiple mutations in cyclophilins and in one FKBP grow, mate, and sporulate normally at 30°C (102, 163). It may be that PPI activity is not essential in yeast. Indeed, PPIs are not

required for folding in vitro, but simply enhance the rate at which folding occurs (63, 226). Alternatively, a high level of redundancy in yeast may cover the loss of even several proteins. At least one other cyclophilin gene and three other FKBP genes are known in yeast (101; J Thorner, personal communication).

The yeast mutations do, however, support the importance of PPIs during exposure to stress. A mutation in the mitochondrial cyclophilin encoded by *CPR3* cannot grow on lactose at 37°C (45). Two other PPIs, the cytoplasmic cyclophilin encoded by *CYP1*, and the ER cyclophilin encoded by *CYP2*, are inducible by heat. Mutations in either or both of these proteins cause cells to die five times more rapidly than wild-type cells at 48°C (252). Critical substrates for these proteins have not yet been identified.

An important recent discovery is that PPIs are present in complexes with two other hsps, hsp70 and hsp90, together with a variety of steroid hormone receptors (179, 254, 255). The FKBP found in these complexes is heat-inducible and is therefore co-regulated with hsp90 and hsp70. (Recall that the FKBPs are an entirely different family of PPIs from the cyclophilins, characterized as heat-inducible in yeast, strengthening the relationship between PPIs and heat tolerance.) The addition of FK506 potentiates the induction of glucocorticoid receptor at low hormone concentrations in cultured cells (186). It is tempting to speculate that the function of PPIs in these complexes is related to the unusual character of hsp90/target protein associations. That is, spontaneous prolyl isomerizations may occur in target proteins while they are bound to hsp90 for extended periods. The speculation can be extended to provide a possible explanation for the importance of PPIs during exposure to extreme temperatures, a circumstance in which unfolded substrates are likely to associate with chaperone proteins for extended periods.

To date, however, there is no direct evidence that any of these mutant phenotypes or drug effects are due to the loss of prolyl isomerase activity. The surprising finding that the immunosuppressive functions of CsA and FK506 are not due to the inhibition of isomerase activity counsels cautious interpretation. Moreover, cyclophilin exhibits a modest chaperone activity with carbonic anhydrase (reducing aggregation during folding in vitro) that is separable from its prolyl isomerase activity (69) and may play a role in folding in vivo. Site-directed mutations that block prolyl isomerase activity (based on the known structures of FKBPs and cyclophilins) offer an important tool for future investigations (290).

Hsp100

THE HSP100 PROTEINS   As discussed above (in the Clp protease section), the *E. coli* ClpA and ClpB proteins and the eukaryotic hsp100 proteins are

members of a highly conserved protein family. The heat-inducible members of this family from yeast, plants, and bacteria have higher homology with each other than with the constitutive members of the family, suggesting that they share a conserved heat-related function (92, 241; E Vierling, personal communication). Genetic analysis of the heat-inducible family members from the yeast *S. cerevisiae* and the bacterium *E. coli* confirm this prediction.

HSP100 FUNCTIONS IN STRESS TOLERANCE   When yeast cells grown at 25°C are pretreated at 37°C to induce tolerance, they survive exposure to 50°C 1000- to 10,000-fold better than non pretreated cells. Cells carrying mutations in the *HSP104* gene also display increased tolerance, but it is very transient. Within a few minutes of exposure to 50°C, mutant cells begin to die at 100 to 1000 times the rate of wild-type cells (218). Hsp104 is specialized to function under extreme conditions. The mutations have no effect on growth on glucose at either low (25°C) or high (39°C) temperatures. At lower killing temperatures (e.g. 44°C), where cells die much more slowly, the Hsp104 protein is less important for tolerance. Here, mutant cells die at ten times the rate of wild-type cells (220). Like *hsp104* mutations, mutations in the heat-inducible *E. coli* gene, *clpB*, have no effect on growth at normal temperatures, but cause cells to die more rapidly than wild-type cells at extreme temperatures (242). Thus, the tolerance functions of the heat-inducible members of this protein family have been conserved.

As described earlier, hsps are induced by a wide variety of toxic conditions, and the hsp100 proteins are no exception. However, the importance of Hsp104 in providing tolerance under different stress conditions varies dramatically. Mutations in the *HSP104* gene have no effect on survival during exposure to copper and cadmium, and have only a minor detrimental effect on survival during exposure to arsenite (220). This suggests that the damage caused by these agents is fundamentally different from the damage caused by heat. Either the Hsp104 protein cannot repair this damage or, if it can, other proteins must be capable of repairing it in the absence of Hsp104. The Hsp104 protein does, however, play a major role in the tolerance of yeast cells to ethanol. As is the case with heat, this function is most apparent under extreme conditions. The *hsp104* mutation reduces tolerance to 20% ethanol by 1000-fold, but reduces tolerance to 15% ethanol by only a few fold (220).

To date, the biochemical function of hsp100 proteins is unknown. Electron micrographs of mutant and wild-type yeast cells exposed to high temperatures reveal the accumulation of large aggregates in the mutant cells, just prior to killing (A Kowal, Y Sanchez, DA Parsell, & S Lindquist, unpublished). It seems likely that this damage is responsible for the death of the mutant cells. However, it is not clear whether the Hsp104 protein normally functions

to prevent these aggregates from forming or to dissolve them once they have formed. As discussed above, a constitutive member of the family, the *E. coli* ClpA protein, functions as a regulator of the ClpP protease (117), but attempts to discern a proteolytic function for the heat-inducible members of the family have failed to date (197, 280).

Genetic analysis of the relationship between hsp70 and Hsp104 provides a clue to Hsp104 function (219). The phenotypes of Hsp104 and hsp70 mutants initially provided no indication of related functions. The *hsp104* mutations have no effect on growth, but drastically reduce survival at extreme temperatures. On the other hand, mutations in the heat-inducible yeast hsp70 genes, *SSA1, SSA3,* and *SSA4,* do not reduce survival at extreme temperatures, but prevent growth at 37°C. Removing *HSP104,* however, uncovers a function for the hsp70 proteins in tolerance. In the *hsp104* mutant strain, *ssa1, ssa3,* and *ssa4,* mutations eliminate most of the residual thermotolerance that is observed in the first few minutes of exposure to 50°C. Furthermore, overexpression of hsp70 increases tolerance in *hsp104* cells, partially compensating for the loss of Hsp104. Conversely, mutations in the constitutively expressed hsp70 genes, *ssa1* and *ssa2,* uncover a function for Hsp104 in growth. The *ssa1ssa2* mutant overexpresses Hsp104 at normal temperatures, and mutation of *hsp104* in this background dramatically reduces the rate of growth. Thus, the Hsp104 protein partially compensates for reduced levels of hsp70 in growth, and the hsp70 proteins partially compensate for hsp104 in thermotolerance (219). These results strongly suggest that Hsp70 and Hsp104 are functioning on the same pathway or on parallel pathways that partially overlap. The Hsp104 protein may promote the proteolysis of damaged proteins that are not salvagable by hsp70. Alternatively, Hsp104 might serve as a molecular chaperone, binding to damaged proteins and promoting refolding. As discussed above, chaperone functions of many hsps may overlap with their proteolytic functions.

CONCLUDING REMARKS

The role hsps play in protecting cells and organisms from stress is complex. Broadly defined, hsps prevent the accumulation of aberrant proteins generated as a result of exposure to high temperatures or other forms of stress. As we have discussed, a large body of evidence points to two general ways in which hsps perform this function. First, certain hsps enhance the flow of substrates through proteolytic pathways known to degrade structurally aberrant proteins. A subset of these hsps are themselves proteases, others are auxiliary components involved in substrate recognition, and the precise role of others is still undefined. Second, hsps function as molecular chaperones, preventing aggregation and restoring the native structures and ac-

tivities of their substrates. These chaperone functions depend upon the ability of hsps to bind to determinants, normally buried within the native structures of proteins, that become exposed when these proteins are damaged by stress. Even within the same general class (i.e. chaperones), the roles of different proteins in stress tolerance varies dramatically among organisms.

The relative importance of refolding and degradation in the cellular response to stress is a major unresolved issue. While mutations in many hsps with known chaperone functions have severe effects on growth and viability, the effects of mutations in proteolytic components are often much more subtle. For example, in *E. coli*, strains carrying a deletion of *dnaK* grow poorly at 30°C and not at all at high (40°C) and low (11–16°C) temperatures. However, strains carrying *lon* and *clpP* mutations are neither temperature-sensitive for growth nor less thermotolerant than wild-type cells. These differences in stress phenotypes might indicate that degradation, in general, plays a smaller role in coping with damaged proteins after a heat stress.

Another possibility is that proteins denatured by heat are degraded by other proteases. Work has focused on Lon and Clp because these proteases are heat-inducible and because they are the major proteases responsible for the degradation of amino-acid analog-containing proteins. Amino-acid analogs (e.g. canavanine) cause misfolding of newly synthesized proteins when they are incorporated in place of natural amino acids (e.g. arginine), and are considered a good model for stress-damaged proteins, in part, because they are strong inducers of hsps. However, the large defect in the degradation of analog-containing proteins in Lon and Clp mutants contrasts sharply with the absence of heat-related phenotypes. Perhaps heat-damaged proteins are different enough in "appearance" from analog-containing proteins that they are efficiently degraded by other proteases, dampening the manifestation of stress-related phenotypes of the *lon* and *clp* mutations.

Clearly, cells do respond to stress by increasing their degradative activities, and it is therefore reasonable to expect these activities to be directed at eliminating stress-damaged proteins. Furthermore, as we have discussed, there are some cases in which mutations in heat-inducible proteolytic components do result in stress-sensitive phenotypes. An important problem for future study is to understand how frequently cells exercise their option to degrade damaged proteins, rather than to salvage them, after they are stressed.

From an energy standpoint, the choice to degrade a stress-damaged protein, rather than to refold it, is an expensive one. Not only is the energy used to synthesize the protein lost (1 ATP and 3 GTP molecules per peptide bond (272)), but additional energy must be employed for its degradation. The vast majority of intracellular proteolysis is ATP-dependent. Lon, for

example utilizes four molecules of ATP per peptide bond cleaved (for review, see (158)). Additional energy is required in eukaryotic systems by enzymes involved in targeting substrates for turnover. On the other hand, salvaging a stress-damaged protein expends a relatively small amount of energy. GroEL, for example, utilizes 130 molecules of ATP per molecule of rhodanese salvaged (157). Renaturing a stress-damaged protein saves the large energetic cost of degrading and resynthesizing it. It makes sense then that cells would salvage stress-damaged proteins when possible. Understanding how a cell determines the level or type of damage that warrants degradation rather than salvage is a question for future investigation.

Organisms appear to respond to different levels of stress by employing the activities of different heat-shock proteins. *E. coli* and *S. cerevisiae* require increased expression of hsp70 for growth at temperatures near the upper end of their normal ranges. Somewhat more severe conditions, such as chronic exposure to temperatures just beyond the upper limit of the normal growth range, require the activities of different proteins (i.e. ubiquitin in *S. cerevisiae*). The most extreme stresses, such as brief exposure to very high temperatures or high concentrations of ethanol, require still other proteins. To survive extreme conditions, both *E. coli* and *S. cerevisiae* require hsp100 proteins. Thus, hsps appear to be specialized in such a way that organisms can appropriately respond to a particular level of stress.

Hsps also appear to be specialized to respond to damage from different types of stress. Consider the response of *S. cerevisiae* to various conditions of extreme stress. The Hsp104 protein is crucial for tolerance to high temperatures and high concentration of ethanol, moderately important for tolerance to arsenite to lower temperatures, and to lower concentrations of ethanol, but of no importance at all for tolerance to copper and cadmium (220). The stress-inducible E2 enzymes appear to be similarly specialized. Ubc4 and Ubc5 play important roles in tolerance to both heat and cadmium, while the effect of Ubc7 is limited to cadmium tolerance (116). Understanding how hsps differentially recognize substrates damaged by various types of stress is crucial for understanding how hsps function to provide stress tolerance.

One of the most perplexing aspects of hsp function in tolerance is that different organisms employ different hsps in response to what would appear to be similar levels of stress. For example, hsp70 is very important for tolerance to killing temperatures in mammals and Drosophila, whereas it plays only a minor role in yeast and *E. coli*. Similarly, members of the hsp100 family are very important for surviving extreme stress in yeast, somewhat important in *E. coli*, perhaps important in mammals, but apparently not important at all in Drosophila. In fact, Drosophila does not even synthesize a hsp100 protein in response to heat shock. Finally, small hsps

promote stress tolerance in mammalian cells, but appear to have little or no effect on tolerance in yeast. Detailed biochemical knowledge of hsp function needs to be integrated with an understanding of the unique physiology of particular organisms.

The diversity of hsps employed in stress tolerance may reflect the different sensitivities of different targets to damage in different organisms. Evidence suggests that nascent chains and folding intermediates of newly synthesized proteins are much more prone to aggregation than proteins that have already achieved their native conformations. Thus, the degree to which normal protein synthesis is repressed in an organism undergoing a heat stress might shift the balance in tolerance functions from one hsp to another. Indeed, organisms differ dramatically in this respect. In logarithmically growing *E. coli* cells, which double their mass every 20 minutes, a substantial fraction of total protein will be present as nascent chains and folding intermediates. *E. coli* cells do not strongly repress normal protein synthesis with standard heat shock, and, given the sensitivity of nascent chains and folding intermediates to heat, newly synthesized proteins may represent the major target of heat damage in *E. coli*. In Drosophila cells, however, the synthesis of constitutive proteins is virtually eliminated during heat shock. Therefore, pre-existing proteins or other cellular components must be the main target of heat damage in this organism. Thus, the lethal lesions incurred during stress by *E. coli* and Drosophila are probably different and might best be repaired by different hsps.

Another major difference between organisms is the basis of cellular architecture. Hsps may play specialized roles in different cell types in protecting large structural complexes from disruption by stress. In certain mammalian cells, for example, one major effect of heat shock is the collapse of the cytoskeletal network. Cells that overexpress small hsps, however, are much less susceptible to this collapse, suggesting that smhsps may function in stress tolerance by preserving the integrity of microfilament structures during stress. It is interesting that in yeast, where smhsps are not especially important for tolerance, microfilament networks are much less elaborate, and the defining feature for cell shape is the cell wall.

In other cases, metabolic constraints may dictate which hsps will be employed for tolerance in a given organism. In *S. cerevisiae*, *hsp104* mutants have a subtle but surprising phenotype: under conditions of respiratory metabolism, they grow slightly faster than wild-type cells (220). If hsp100 proteins are slightly deleterious to respiring cells, in an organism like Drosophila, where a large percentage of total mass is devoted to flight muscle, this might provide evolutionary pressure to find another avenue to stress tolerance. The solution, apparently, has been to amplify hsp70 genes and to evolve a complex series of regulatory mechanisms that allow an

enormous induction of hsp70 in response to stress. That hsp70 has the ability to fulfill such a role, even in yeast, is demonstrated by the fact that overexpression of hsp70 partially compensates for the loss of Hsp104 in thermotolerance (219). With different levels of various hsps, organisms apparently achieve a balance of activities ideally suited to their own normal physiological states and to the types of stress conditions to which they are subject.

Finally, while this review has stressed the function of heat-inducible proteins in the cellular response to stress, it is important to remember that many other factors also are likely to play a role in stress tolerance. Trehalose, for example, is a disaccharide whose accumulation in *S. cerevisiae* closely parallels acquisition of a stress-tolerant state. It is possible that trehalose directly promotes the reactivation of stress-damaged proteins. In vitro studies show that labile enzymes dried in the presence of trehalose can be reconstituted after prolonged storage with no loss of activity and can withstand exposure to extreme heat (39).

Other small molecules like glycerol, betaine, and proline accumulate in various organisms under stress-tolerant conditions and may play a similar role. These molecules function as osmolytes, protecting organisms from "water stress", (i.e. changing salinity, dessication, or freezing) (284). Surprisingly, cartilaginous marine fishes accumulate urea in their blood and cells to a concentration of 400 mM. The proteins of these organisms are as susceptible to unfolding by urea as are proteins of other organisms, and how they remain active in its presence was a puzzle. These organisms also accumulate methylamines like trimethylamine-N-oxide (TMAO), betaine, and sarcosine, which act to counter the effects of urea (reviewed in (284)), resolving the puzzle. In fact, in vitro studies show that these molecules counter the deleterious effects of urea on the activities of several enzymes and, by themselves, increase the melting temperature of ribonuclease. It is not yet clear whether these small molecules stabilize protein structures by interacting with them directly or by affecting the solvent properties of nearby water molecules (284). Whatever the mechanism, it is likely that small organic molecules and inorganic ions play an important role in protecting cellular proteins that become denatured during conditions of stress.

Posttranslational modification might play an equally important role in preventing protein aggregation and promoting proper folding. As discussed above, hsp70 and hsp60 proteins are very important for the folding of proteins in bacteria, mitochondria, and chloroplasts. Related proteins (hsc70 and TRiC) are important in the eukaryotic cytosol. Proteins related to hsp70 are present in the ER and are required for protein translocation into that compartment. Strangely, however, no hsp60 family member has been localized to the ER/Golgi compartment. Proteins in this compartment are

characterized by two posttranslational modifications that are rare in other compartments, glycosylation and disulfide bond formation. It has been suggested that glycosylation protects ER proteins from aggregation, in effect, mimicking the action of a protein chaperone (120, 121). Similarly, many in vitro studies show that disulfide bonds stabilize protein structures to high temperatures, and might obviate the need for molecular chaperones. Another possibility is that non heat-inducible proteins function as chaperones in the ER and Golgi (189a).

Attempts to understand precisely how hsps function at high temperatures are stymied by how little we know about the physical characteristics of stress-damaged proteins (50). Do these proteins expose extended stretches of unstructured polypeptide chain or are their structures more compact? To what extent are intracellular stress-damaged proteins aggregated? These become central questions now that the biochemical activities of many hsps are being studied in vitro. The answers to these questions will aid the design of experiments to investigate the physiologically relevant functions of hsps in stress tolerance. Chaperones and proteases might act purely to prevent the formation of protein aggregates or they might act to dissolve them. Preliminary data suggest that cells can reactivate some proteins even once they have formed massive aggregates. When Xenopus vimentin was expressed in mammalian cells at 37°C, it aggregated into large structures that were easily visible by electron microscopy (107). Yet, when these cells were shifted to 28°C, the vimentin was seen to spool out from the aggregates and to begin to form normal-looking vimentin filaments. Even among the set of heat-damaged aggregated proteins that are renatured, there may exist differences in structure that influence the activities of the chaperones that repair them.

Nature has discovered many different ways to meet the challenge of a changing environment. Over the last five to six years, we have learned a great deal about the biochemical activities of hsps, their interactions with each other, and with their targets. The next challenge will be to place this knowledge within the physiological context of particular types of cells and specific organisms.

Literature Cited

1. Abernethy RH, Thiel DS, Petersen NS, Helm K. 1989. Thermotolerance is developmentally dependent in germinating wheat seeds. *Plant Physiol.* 89:569–79

1a. Atkinson BG, Walden DB, eds. 1985. *Changes in Eukaryotic Gene Expression in Response to Environmental Stress.* Orlando, FL: Academic

2. Amerik AY, Antonov VK, Gorbalenya AE, Kotova SA, Rotanova TV, et al. 1991. Site-directed mutagenesis of La protease. *FEBS Lett.* 287:211–14

3. Ananthan J, Goldberg AL, Voellmy R. 1986. Abnormal proteins serve as eukaryotic stress signals and trigger the activation of heat shock genes. *Science* 232:522–24

4. Anderson RL, Van KI, Kraft PE, Hahn GM. 1989. Biochemical analysis of heat-resistant mouse tumor cell strains: a new member of the HSP70 family. *Mol. Cell Biol.* 9:3509–16

5. Angelidis CE, Lazaridis I, Pagoulatos GN. 1991. Constitutive expression of heat-shock protein 70 in mammalian cells confers thermoresistance. *Eur. J. Biochem.* 199:35–39

5a. Aoyama A, Frohli E, Schafer R, Klemenz R. 1993. αβ-Crystallin expression in mouseNIH 3T3 fibroblasts: glucocorticoid responsiveness and involvement in thermal protection. *Mol. Cell. Biol.* 13:1824–35

6. Arrigo AP, Tanguay RM. 1991. Expression of heat shock proteins during development in Drosophila. *Results Probl. Cell Differ.* 17:106–19

7. Baker TA, Grossman AD, Gross CA. 1984. A gene regulating the heat shock response in *Escherichia coli* also affects proteolysis. *Proc. Natl. Acad. Sci. USA* 81:6779–83

8. Banerji J, Sands J, Strominger JL, Spies T. 1990. A gene pair from the human major histocompatibility complex encodes large proline-rich proteins with multiple repeated motifs and a single ubiquitin-like domain. *Proc. Natl. Acad. Sci. USA* 87:2374–78

9. Bansal GS, Norton PM, Latchman DS. 1991. The 90-kDa heat shock protein protects mammalian cells from thermal stress but not from viral infection. *Exp. Cell Res.* 195:303–6

10. Bardwell JC, Craig EA. 1988. Ancient heat shock gene is dispensable (published erratum appears in *J. Bacteriol.* 1988 170:4999). *J. Bacteriol.* 170: 2977–83

11. Barraclough R, Ellis RJ. 1980. Protein synthesis in chloroplasts IX. Assembly of newly synthesized large subunits into ribulose bisphosphate carboxylase in isolated contact pea chloroplasts. *Biochem. Biophys. Acta* 608:19–31

12. Bartel B, Wunning I, Varshavsky A. 1990. The recognition component of the N-end rule pathway. *EMBO J.* 9:3179–89

13. Beckmann RP, Mizzen LE, Welch WJ. 1990. Interaction of hsp70 with newly synthesized proteins: implications for protein folding and assembly. *Science* 248:850–54

14. Bierer BE, Mattila PS, Standaert RF, Herzenberg LA, Burakoff SJ, et al. 1990. Two distinct signal transmission pathways in T lymphocytes are inhibited by complexes formed between an immunophilin and either FK506 or rapamycin. *Proc. Natl. Acad. Sci. USA* 87:9231–35

15. Bierer BE, Sommers PK, Wandless TJ, Burakoff SJ, Schreiber SL. 1990. Probing immunosuppressant action with a nonnatural immunophilin ligand. *Science* 250:556–59

16. Bird P, Gething M-J, Sambrook J. 1987. Translocation in yeast and mammalian cells: not all signal sequences are functionally equivalent. *J. Cell Biol.* 105:2905–14

17. Blond-Elguindi S, Fourie AM,

Sambrook JF, Gething M-J, et al. 1993. Peptide-dependence of the ATPase activity of the molecular chaperone BiP is the result of conversion of oligomers to active monomers. *J. Biol. Chem.* In press

18. Bohley P, Seglen PO. 1992. Proteases and proteolysis in the lysosome. *Experientia* 48:151–57

19. Bond U, Schlesinger MJ. 1985. Ubiquitin is a heat shock protein in chicken embryo fibroblasts. *Mol. Cell Biol.* 5:949–56

20. Boon-Niermeijer EK. 1991. Heat shock effects in snail development. *Results Probl. Cell Differ.* 17:7–28

21. Bork P, Sander C, Valencia A, Bukau B. 1992. A module of the DnaJ heat shock proteins found in malaria parasites. *Trends Biochem. Sci.* 17:129

22. Borkovich KA, Farrelly FW, Finkelstein DB, Taulien J, Lindquist S. 1989. Hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher temperatures. *Mol. Cell Biol.* 9:3919–30

23. Deleted in proof

24. Browder LW, Pollock RM, Neckells RW, Heikkila JJ, Winning RS. 1988. Developmental regulation of the heat shock response. In *Genomic Adaptability in Cell Specialization*, ed. MA Bernardino, L Etrin, pp. 97–147. New York: Plenum

25. Brugge JS. 1986. Interaction of the Rous sarcoma virus protein pp60src with the cellular proteins pp50 and pp90. *Curr. Top. Microbiol. Immunol.* 123:1–22

26. Brunschier R, Danner M, Seckler R. 1993. Interactions of phage P22 tailspike protein with GroE molecular chaperones during refolding in vitro. *J. Biol. Chem.* 268:2767–72

27. Brunt SA, Riehl R, Silver JC. 1990. Steroid hormone regulation of the *Achlya ambisexualis* 85-kilodalton heat shock protein, a component of the Achlya steroid receptor complex. *Mol. Cell Biol.* 10:273–81

28. Bukau B. 1993. Regulation of the *E. coli* heat shock response. *Mol. Microbiol.* 9:671–80

29. Bukau B, Walker GC. 1989. Cellular defects caused by deletion of the *Escherichia coli dnaK* gene indicate roles for heat shock protein in normal metabolism. *J. Bacteriol.* 171:2337–46

30. Bukau B, Walker GC. 1990. Mutations altering heat shock specific subunit of RNA polymerase suppress major cellular defects of *E. coli* mutants lacking

the DnaK chaperone. *EMBO J.* 9:4027–36

31. Butt TR, Jonnalagadda S, Monia B, Sternberg EJ, Marsh JA, et al. 1989. Ubiquitin fusion augments the yield of cloned gene products in *Escherichia coli. Proc. Natl. Acad. Sci. USA* 86:2540–44

32. Caplan AJ, Cyr DM, Douglas MG. 1992. YDJ1p facilitates polypeptide translocation across different intracellular membranes by a conserved mechanism. *Cell* 71:1143–55

32a. Caplan AJ, Cyr DM, Douglas MG. 1993. Eukaryotic homologs of *Escherichia coli* dnaJ: a diverse protein family that functions with HSP70 stress proteins. *Mol. Biol. Cell* 4:555–63

33. Carper SW, Duffy JJ, Gerner EW. 1987. Heat shock proteins in thermotolerance and other cellular processes. *Cancer Res.* 47:5249–55

34. Chandresekhar GN, Tilly K, Woolford C, Hendrix R, Georgopoulos C. 1986. Purification and properties of the GroES morphogenetic protein of *Escherichia coli. J. Biol. Chem.* 261:12414–19

35. Chang SC, Erwin E, Lee AS. 1989. Glucose-regulated protein (*GRP94* and *GRP78*) genes share common regulatory domains and are coordinately regulated by common trans-acting factors. *Mol. Cell. Biol.* 9:2153–62

36. Cheng MY, Hartl FU, Martin J, Pollock RA, Kalousek F, et al. 1989. Mitochondrial heat-shock protein hsp60 is essential for assembly of proteins imported into yeast mitochondria. *Nature* 337:620–25

37. Chin DT, Goff SA, Webster T, Smith T, Goldberg AL. 1988. Sequence of the *lon* gene in *Escherichia coli*: A heat-shock gene which encodes the ATP-dependent protease La. *J. Biol. Chem.* 263:11718–28

37a. Chirico WJ, Waters MG, Blobel G. 1988. 70K heat shock-related proteins stimulate protein translocation into microsomes. *Nature* 332:805–10

38. Chretien P, Landry J. 1988. Enhanced constitutive expression of the 27-kDa heat shock proteins in heat-resistant variants from Chinese hamster cells. *J. Cell Physiol.* 137:157–66

39. Colaco C, Sen S, Thangavelu M, Pinder S, Roser B. 1992. Extraordinary stability of enzymes dried in trehalose: simplified molecular biology. *Biotechnology* 10:1007–11

40. Craig E, Kang PJ, Boorstein W. 1990. A review of the role of 70 kDa heat shock proteins in protein translocation

across membranes. *Antonie Van Leeuwenhoek* 58:137–46

41. Craig EA, Gross CA. 1991. Is hsp70 the cellular thermometer? *Trends in Biochem. Sci.* 16:135–40

42. Craig EA, Jacobsen K. 1984. Mutations of the heat inducible 70 kilodalton genes of yeast confer temperature sensitive growth. *Cell* 38:341–49

43. Craig EA, Jacobsen K. 1985. Mutations in cognate genes of *Saccharomyces cerevisiae* hsp70 result in reduced growth rates at low temperatures. *Mol. Cell Biol.* 5:3517–24

44. Csermely P, Kahn CR. 1991. The 90-kDa heat shock protein (hsp-90) possesses an ATP binding site and autophosphorylating activity. *J. Biol. Chem.* 266:4943–50

45. Davis ES, Becker A, Heitman J, Hall MN, Brennan MB. 1993. A novel yeast cyclophilin gene essential for lactate metabolism at high temperature. *Proc. Natl. Acad. Sci. USA* 89:11169–73

46. DeLuca-Flaherty C, McKay DB, Parham P, Hill BL. 1990. Uncoating protein (hsc70) binds a conformationally labile domain of clathrin light chain LCa to stimulate ATP hydrolysis. *Cell* 62:875–87

47. Deshaies RJ, Koch BD, Werner-Washburne M, Craig EA, Schekman R. 1988. A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor proteins. *Nature* 332:800–5

48. Dice JF. 1990. Peptide sequences that target cytosolic proteins for lysosomal proteolysis. *Trends Biochem. Sci.* 15:305–9

49. DiDomenico BJ, Bugaisky GE, Lindquist S. 1982. The heat shock response is self-regulated at both the transcriptional and posttranscriptional levels. *Cell* 31:593–603

50. Dill KA, Shortle D. 1991. Denatured states of proteins. *Annu. Rev. Biochem.* 60:795–825

51. Doherty FJ, Osborn N, Wassell JA, Heggie PE, Laszio L. 1989. Ubiquitin-protein conjugates accumulate in the lysosomal system of fibroblasts treated with cysteine proteinase inhibitors. *Biochem. J.* 263:47–55

52. Dubiel W, Ferrell K, Pratt G, Rechsteiner M. 1992. Subunit 4 of the 26S protease is a member of a novel eukaryotic ATPase family. *J. Biol. Chem.* 267:22699–702

53. Dura JM. 1981. Stage dependent synthesis of heat shock induced proteins in early embryos of *Drosophila*

melanogaster. Mol. Gen. Genet. 184:381–85

54. Ecker D, Stadel JM, Butt TR, Marsh JA, Monia BP, et al. 1989. Increasing gene expression in yeast by fusion to ubiquitin. *J. Biol. Chem.* 264:7715–19

55. Ellis, RJ. 1993. The general concept of molecular chaperones. *Philos. Trans. R.Soc. London Ser B.* 339:257–62

56. Ellis RJ, van der Vies SM. 1991. Molecular chaperones. *Annu. Rev. Biochem.* 60:321–47

57. Erdmann R, Wiebel FF, Flessau A, Rytka J, Beyer A, et al. 1991. PAS1, a yeast gene required for peroxisome biogenesis, encodes a member of a novel family of putative ATPases. *Cell* 64:499–510

58. Fayet O, Ziegelhoffer T, Georgopoulos C. 1989. The groES and groEL heat shock gene products of *Escherichia coli* are essential for bacterial growth at all temperatures. *J. Bacteriol.* 171:1379–85

59. Feder JH, Rossi JM, Solomon J, Solomon N, Lindquist S. 1992. The consequences of expressing hsp70 in Drosophila cells at normal temperatures. *Genes Dev.* 6:1402–13

60. Finley D, Bartel B, Varshavsky A. 1989. The tails of ubiquitin precursors are ribosomal proteins whose fusion to ubiquitin facilitates ribosome biogenesis. *Nature* 338:394–401

61. Finley D, Chau V. 1991. Ubiquitination. *Annu. Rev. Cell. Biol.* 7:25–69

62. Finley D, Ozkaynak E, Varshavsky A. 1987. The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation, and other stresses. *Cell* 37:1035–46

63. Fischer G, Bang H, Mech C. 1984. Detection of enzyme catalysis for cis-trans isomerization of peptide bonds using proline containing peptides as substrates. *Biomed. Biochim. Acta* 43:1101–12

64. Flaherty KM, DeLuca-Flaherty C, McKay DB. 1990. Three-dimensional structure of the ATPase fragment of a 70K heat-shock cognate protein (see comments). *Nature* 346:623–28

65. Flynn GC, Pohl J, Flocco MT, Rothman JE. 1991. Peptide-binding specificity of the molecular chaperone BiP. *Nature* 353:726–30

66. Folz KR, Partin JS, Lennarz WJ. 1993. Sea urchin egg receptor for sperm: sequence similarity of binding domain and hsp70. *Science* 259:1421–25

67. Fostinis Y, Theodoropoulos PA, Gravanis A, Stournaras C. 1992. Heat shock protein HSP90 and its association

with the cytoskeleton: a morphological study. *Biochem. Cell Biol.* 70:779–86

68. Freedman RB. 1992. Protein folding in the cell. in *Protein Folding,* ed. TE Creighton, pp. 457–541. New York: Freeman

69. Freskgard P-O, Bergenhem N, Jonsson B-H, Svensson M, Carlsson U. 1992. Isomerase and chaperone activity of prolyl isomerase in the folding of carbonic anhydrase. *Science* 258:466–68

70. Frohlich K-U, Fries H-W, Rudiger M, Erdmann R, Botstein D. 1991. Yeast cell cycle protein CDC48p shows full-length homology to the mammalian protein VCP and is a member of a protein family involved in secretion, perosisome formation, and gene expression. *J. Cell Biol.* 114:443–53

71. Frydman J, Nimmesgern E, Erdjument BH, Wall JS, Tempst P, et al. 1992. Function in protein folding of TRiC, a cytosolic ring complex containing TCP-1 and structurally related subunits. *EMBO J.* 11:4767–78

72. Gamer J, Bujard H, Bukau B. 1992. Physical interaction between heat shock proteins DnaK, DnaJ, GrpE and the bacterial heat shock transcription factor sigma 32. *Cell* 69:833–42

73. Gao Y, Thomas JO, Chow RL, Lee GH, Cowan NJ. 1992. A cytoplasmic chaperonin that catalyzes beta-actin folding. *Cell* 69:1043–50

74. Gao Y, Vainberg IE, Chow RL, Cowan NJ. 1993. Two cofactors and cytoplasmic chaperonin are required for the folding of α- and β-tubulin. *Mol. Cell. Biol.* 13:2478–85

75. Gaut JR, Hendershot LM. 1993. Mutations within the nucleotide binding site of BiP inhibit ATPase activity and interfere with release of immunoglobulin heavy chain. *J. Biol. Chem.* 268:7248–55

76. Georgopoulos C, Ang D. 1990. The *Escherichia coli* groE chaperonins. *Semin. Cell Biol.* 1:19–25

77. Georgopoulos C, Ang D, Liberek K, Zylicz M.et al. 1990. Properties of the *Escherichia coli* heat shock proteins and their role in bacteriophase λ growth. In *Stress Proteins in Biology and Medicine,* ed. RI Morimoto, A Tissieres, C. Georgopoulos, pp. 191–221. Cold Spring Harbor, NY: Cold Spring Harbor Lab. Press

78. Gething MJ, McCammon K, Sambrook J. 1986. Expression of wild-type and mutant forms of influenza hemagglutinin: the role of folding in intracellular transport. *Cell* 46:939–50

79. Gething MJ, Sambrook J. 1992. Protein folding in the cell. *Nature* 355:33–45

80. Glotzer M, Murray AW, Kirschner MW. 1991. Cyclin is degraded by the ubiquitin pathway. *Nature* 349:132–38

81. Goff SA, Casson LP, Goldberg AL. 1984. Heat shock regulatory gene *htpR* influences rates of protein degradation and expression of the *lon* gene in *Escherichia coli. Proc. Natl. Acad. Sci. USA* 81:6647–51

82. Goff SA, Goldberg AL. 1985. Production of abnormal proteins in *E. coli* stimulates transcription of *lon* and other heat shock genes. *Cell* 41:587–95

83. Goff SA, Voellmy R, Goldberg AL, 1988. Protein breakdown and the heat-shock response. In *Ubiquitin,* ed. M Rechsteiner, pp. 207–38. New York: Plenum

83a. Goldberg AL. 1972. Degradation of abnormal proteins in *Esherichia Coli. Proc. Natl.* Acad. Sci. USA 69:422–26

84. Goldberg AL, Rock KL. 1992. Proteolysis, proteosomes and antigen presentation. *Nature* 357:375–79

85. Goloubinoff P, Gatenby AA, Lorimer GH. 1989. GroE heat-shock proteins promote assembly of foreign prokaryotic ribulose bisphosphate carboxylase oligomers in *Escherichia coli. Nature* 337:44–47

86. Deleted in proof

87. Gordon CL, King J. 1993. Temperature-sensitive mutations in the phage P22 coat protein which interfere with polypeptide chain folding. *J. Biol. Chem.* 268:9358–68

88. Gottesman S. 1989. Genetics of proteolysis in *Escherichia coli. Annu. Rev. Genet.* 23:163–98

89. Gottesman S, Clark WP, de Crecy-Lagard V, Maurizi MR. 1993. ClpX, an alternative subunit for the ATP-dependent Clp protease of *Escherichia coli:* sequence and in vivo activities. *J. Biol. Chem.* In press

90. Gottesman S, Clark WP, Maurizi MR. 1990. The ATP-dependent Clp protease of *Escherichia coli:* sequence of *clpA* and identification of a Clp-specific substrate. *J. Biol. Chem.* 265:7886–93

91. Gottesman S, Maurizi MR. 1992. Regulation by proteolysis: energy-dependent proteases and their targets. *Microbiol. Rev.* 56:592–621

92. Gottesman S, Squires C, Pichersky E, Carrington M, Hobbs M, et al. 1990. Conservation of the regulatory subunit for the Clp ATP-dependent protease in prokaryotes and eukaryotes. *Proc. Natl. Acad. Sci. USA* 87:3513–17

93. Graziosi G, Cristinii FD, Marcotullio

AD, Marzari R, Micali F, et al. 1983. Morphological and molecular modifications induced by heat shock in Drosophila melanogaster embryos. *J. Embryol. Exp. Morphol.* 77:167–82

94. Gropper R, Brandt RA, Elias S, Bearer CF, Mayer A, et al. 1991. The ubiquitin-activating enzyme, E1, is required for stress-induced lysosomal degradation of cellular proteins. *J. Biol. Chem.* 266:3602–10

94a. Gupta RS. 1990. Sequence and structural homology between a mouse T-complex protein TCP-1 and the 'chaperonin' family of bacterial (GroEL, 60–65 kDa heat shock antigen) and the eukaryotic proteins. *Biochem. Int.* 20:833–41

95. Gupta RS, Singh B. 1992. Cloning of the HSP70 gene from *Halobacterium marismortui:* relatedness of archaebacterial HSP70 to its eubacterial homologs and a model for the evolution of the HSP70 gene. *J. Bacteriol.* 174:4594–605

96. Haas AL, Ahrens P, Bright PM, Ankel H. 1987. Interferon induces a 15-kilodalton protein exhibiting marked homology to ubiquitin. *J. Biol. Chem.* 262:11315–23

97. Hahn GM. 1982. Hyperthermia and cancer. In *Thermotolerance: Thermotolerance and Thermophily,* ed. K Henle. pp. 97–112. New York: Plenum

98. Hartl FU, Martin J, Neupert W. 1992. Protein folding in the cell: the role of molecular chaperones Hsp70 and Hsp60. *Annu. Rev. Biophys. Biomol. Struct.* 21:293–322

99. Heikkila JJ, Kloc M, Bury J, Schultz GA, Browder LW. 1985. Acquisition of the heat-shock response and thermotolerance during early development of *Xenopus laevis. Dev. Biol.* 107:483–89

100. Heinemeyer W, Kleinschmidt JA, Saidowsky J, Escher C, Wolf DH. 1991. Proteinase yscE, the yeast proteasome/multicatalytic - multifunctional proteinase: mutants unravel its function in stress induced proteolysis and uncover its necessity for cell survival. *EMBO J.* 10:555–62

101. Heitman J, Movva NR, Hall MN. 1992. Proline isomerases at the crossroads of protein folding, signal transduction, and immunosuppression. *New Biol.* 4:448–60

102. Heitman J, Movva NR, Hiestand PC, Hall MNM. 1991. FK506-binding protein proline rotamase is a target for the immunosuppressive agent FK506

in *Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA* 88:1948–52

103. Hemmingsen SM, Woolford C, van der Vies SM, Tilly K, Dennis DT, et al. 1988. Homologous plant and bacterial proteins chaperone oligomeric protein assembly. *Nature* 333:330–34

103a. Hendrey J, Kola I. 1991. Thermolability of mouse oocytes is due to the lack of expression and/or inducibility of hsp70. *Mol. Reprod. Dev.* 28:1–8

104. Hendrick JP, Langer T, Davis TA, Hartl FU, Wiedman M. 1993. Control of folding and membrane translocation by binding of the chaperone DnaJ to nascent polypeptides. *Proc. Natl. Acad. Sci. USA.* In press

105. Hendrix RW. 1979. Purification and properties of groE, a host protein involved in bacteriophage assembly. *J. Mol. Biol.* 129:375–92

106. Hengge-Aronic R. 1993. Survival of hunger and stress: the role of rpoS in early stationary phase gene regulation in *E. coli. Cell* 72:165–68

107. Herrmann H, Eckelt A, Brettel M, Grund C, Franke WW. 1993. Temperature-sensitive intermediate filament assembly: alternative structures of *Xenopus laevis* vimentin in vitro and in vivo. *J. Mol. Biol.* In press

108. Hershko A, Ciechanover A. 1992. The ubiquitin system for protein degradation. *Annu. Rev. Biochem.* 61:761–807

109. Hochstrasser M, Ellison MJ, Chau V, Varshavsky A. 1991. The short-lived MATα2 transcriptional regulator is ubiquitinated in vivo. *Proc. Natl. Acad. Sci. USA* 88:4606–10

110. Holl-Neugebauer B, Rudolph R, Schmidt M, Buchner J. 1991. Reconstitution of a heat shock effect in vitro: influence of GroE on the thermal aggregation of alpha-glucosidase from yeast. *Biochemistry* 30:11609–14

111. Horwich AL, Willison K. 1993. Protein folding in the cell: Functions of two families of molecular chaperone, hsp60 and TF55-TCP1. *Philos. Trans. R. Soc. London Ser. B.* 339:313–26

112. Hutchison KA, Stancato LF, Jove R, Pratt WB. 1992. The protein-protein complex between pp60v-src and hsp90 is stabilized by molybdate, vanadate, tungstate, and an endogenous cytosolic metal. *J. Biol. Chem* 267:13952–57

113. Jakob U, Gaestel M, Engel K, Buchner J. 1993. Small heat shock proteins are molecular chaperones. *J. Biol. Chem.* 268:1517–20

114. Jentsch S. 1992. The ubiquitin-conju-

gation system. *Annu. Rev. Genet.* 26: 179–207

115. Jentsch S, Sommer T. 1993. A protein translocation defect linked to ubiquitin conjugation at the endoplasmic reticulum. *Nature* 365:176–79

116. Jungmann J, Reins HA, Schobert C, Jentsch S. 1993. Resistance to cadmium mediated by ubiquitin-dependent proteolysis. *Nature* 361:369–71

117. Katayama Y, Gottesman S, Pumphrey J, Rudikoff S, Clark WP, et al. 1988. The two-component, ATP-dependent Clp protease of *Escherichia coli*. *J. Biol. Chem.* 263:15226–36

118. Keller JA, Simon LD. 1988. Divergent effects of a dnaK mutation on abnormal protein degradation in *Escherichia coli*. *Mol. Microbiol.* 2:31–41

119. Deleted in proof

120. Kern G, Schmidt M, Buchner J, Jaenicke R. 1992. Glycosylation inhibits the interaction of invertase with the chaperone GroEL. *FEBS Lett.* 305: 203–5

121. Kern G, Schulke N, Schmid FX, Jaenicke R. 1992. Quaternary structure and stability of internal, external, coreglycosylated invertase from yeast. *Protein Sci.* 1:120–31

122. Kiefhaber T, Rudolph R, Kohler HH, Buchner J. 1991. Protein aggregation in vitro and in vivo: a quantitative model of the kinetic competition between folding and aggregation. *Biotechology* 9:825–29

123. Kim PS, Baldwin RL. 1990. Intermediates in the folding reactions of small proteins. *Annu. Rev. Biochem.* 59:631–60

124. Kimura Y, Matsumoto S, Yahara I. 1993. Temperature-sensitive mutants of hsp82 of the budding yeast, *Saccharomyces cerevisiae*. *Md. Gen. Genet.* In press

125. Klausner RD, Sitia R. 1990. Protein degradation in the endoplasmic reticulum. *Cell* 62:611–14

126. Klemenz R, Hultmark D, Gehring WJ. 1985. Selective translation of heat shock mRNA in *Drosophila melanogaster* depends on sequence information in the leader. *EMBO J.* 4:2053–60

127. Knecht E, Palmer A, Sweeney ST, Rivett AJ. 1991. Immunocytochemical localization of the multicatalytic proteinase in rat liver and in L-132 cells. *J. Biochem. Soc. Trans.* 19:2935

128. Kohno K, Normington K, Sambrook J, Gething M-J, Mori K, et al. 1993. The promoter region of the yeast KAR2 (BiP) gene contains a regulatory do-

main that responds to the presence of unfolded proteins in the endoplasmic reticulum. *Mol. Cell. Biol.* 13:877–90

129. Kroh HE, Simon LD. 1990. The ClpP component of Clp protease is the sigma 32-dependent heat shock protein F21.5. *J. Bacteriol.* 172:6026–34

130. Kusukawa N, Yura T. 1988. Heat shock protein GroE of *Escherichia coli*: key protective roles against thermal stress. *Genes Dev.* 2:874–82

131. Laminet AA, Ziegelhoffer T, Georgopoulos C, Pluckthun A. 1990. The *Escherichia coli* heat shock proteins GroEL and GroES modulate the folding of the beta-lactamase precursor. *EMBO J.* 9:2315–19

132. Landry J. 1992. HSP27 may couple signal transduction pathways to microfilament responses. *J. UOEH* 15:111–21

133. Landry J, Chretien P, Lambert H, Hickey E, Weber LA. 1989. Heat shock resistance conferred by expression of the human HSP27 gene in rodent cells. *J. Cell Biol.* 109:7–15

133a. Landry J, Lambert H, Zhou M, Lavoie JN, Hickey E, et al. 1992. Human HSP27 is phosphorylated at serines 78 and 82 by heat shock and mitogen-activated kinases that recognize the same amino acid motif as S6 kinase II. *J. Biol. Chem.* 267:794–803

134. Landry SJ, Gierasch LM. 1991. The chaperonin GroEL binds a polypeptide in an alpha-helical conformation. *Biochemistry* 30:7359–62

135. Landry SJ, Jordan R, McMacken R, Gierasch LM. 1992. Different conformations for the same polypeptide bound to chaperones DnaK and GroEL. *Nature* 355:455–57

136. Langer T, Lu C, Echols H, Flanagan J, Hayer MK, et al. 1992. Successive action of DnaK, DnaJ, and GroEL along the pathway of chaperone-mediated protein folding. *Nature* 356:683–89

137. Langer T, Pfeifer G, Martin J, Baumeister W, Hartl FU. 1992. Chaperonin-mediated protein folding: GroES binds to one end of the GroEL cylinder, which accommodates the protein substrate within its central cavity. *EMBO J.* 11:4757–65

137a. Laslzo A. 1992. The effects of hyperthermia on mammalian cell structure and function. *Cell Prolif.* 25:59–87

138. Laszlo A, Li GC. 1985. Heat-resistant variants of Chinese hamster fibroblasts altered in expression of heat shock

protein. *Proc. Natl. Acad. Sci. USA* 82:3029–33

139. Lavoie JN, Gingras-Breton G, Tanguay RM, Landry J. 1993. Induction of Chinese hamster HSP27 gene expression in mouse cells confers resistance to heat shock. *J. Biol. Chem.* 268: 3420–29

140. Lee HS, Simon JA, Lis JT. 1988. Structure and expression of ubiquitin genes of *Drosophila melanogaster. Mol. Cell Biol.* 8:4727–35

141. Lewis VA, Hynes GM, Zheng D, Saibil H, Willison K. 1992. T-complex polypeptide-1 is a subunit of a heteromeric particle in the eukaryotic cytosol. *Nature* 358:249–52

142. Li GC, Laszlo A. 1985. Thermotolerance in mammalian cells: a possible role for heat shock proteins. See Ref. 1a, pp. 227–54.

143. Li GC, Li LG, Liu YK, Mak JY, Chen LL, et al. 1991. Thermal response of rat fibroblasts stably transfected with the human 70-kDa heat shock protein-encoding gene. *Proc. Natl. Acad. Sci. USA* 38:1681–85

144. Li GC, Werb Z. 1982. Correlation between synthesis of heat shock proteins and development of thermotolerance in Chinese hamster fibroblasts. *Proc. Natl. Acad. Sci. USA* 79:3218–22

145. Liberek K, Galitski TP, Zylicz M, Georgopoulos C. 1992. The DnaK chaperone modulates the heat shock response of *Escherichia coli* by binding to the sigma 32 transcription factor. *Proc. Natl. Acad. Sci. USA* 89:3516–20

146. Lindquist S. 1980. Translational efficiency of heat-induced messages in *Drosophila melanogaster* cells. *J. Mol. Biol.* 137:151–58

147. Lindquist S. 1986. The heat-shock response. *Annu. Rev. Biochem.* 55: 1151–91

148. Lindquist S. 1992. Heat-shock proteins and stress tolerance in microorganisms. *Curr. Opin. Genet. Dev.* 2:748–55

148a. Lindquist S. 1992. Won't you change partners and dance? Curr. Biol. 2:119–21

149. Lindquist S, Craig EA. 1988. The heat-shock proteins. *Annu. Rev. Genet.* 22:631–77

150. Deleted in proof

151. Lindquist S, DiDomenico B. 1985. Coordinate and noncoordinate gene expression during heat shock: A model for regulation. See Ref. 1a, pp. 71–89

151a. Lindquist S, Parsell DA, Sanchez Y,

Taulien J, Craig EA, et al. 1993. Heat-shock proteins in stress tolerance. *J. UOEH* 15:1–9

152. Lipinska B, Fayet O, Baird L, Georgopoulos C. 1989. Identification, characterization and mapping of the *Escherichia coli htrA* gene, whose product is essential for bacterial growth only at elevated temperatures. *J. Bacteriol.* 171:1574–84

153. Lipinska B, Zylicz M, Georgopoulos C. 1990. The HtrA (DegP) protein, essential for *Escherichia coli* survival at high temperatures, is an endopeptidase. *J. Bacteriol.* 172:1791–97

154. Lodish HF, Kong N. 1991. Cyclosporin A inhibits an initial step in folding of transferrin within the endoplasmic reticulum. *J. Biol. Chem.* 266:14835–38

155. Lorimer GH. 1992. Role of accessory proteins in protein folding. *Curr. Opin. Struct. Biol.* 2:26–34

156. Martin J, Horwich AL, Hartl FU. 1992. Prevention of protein denaturation under heat stress by the chaperonin Hsp60. *Science* 258:995–98

157. Martin J, Langer T, Boteva R, Schramel A, Horwich AL, et al. 1991. Chaperonin-mediated protein folding at the surface of groEL through a 'molten globule'-like intermediate (see comments). *Nature* 352:36–42

158. Maurizi MR. 1992. Proteases and protein degradation in *Escherichia coli. Experientia* 48:178–201

159. Deleted in proof

160. Maurizi MR, Clark WP, Kim SH, Gottesman S. 1990. ClpP represents a unique family of serine proteases. *J. Biol. Chem.* 265:12546–52

160a. McCarty JS, Walker GC. 1993. Expression of Dnak defective in ATPase activity leads to temperature-dependent filamentation of *Escherichia coli. J. Bacteriol.* Manuscript submitted

161. McGarry TJ, Lindquist S. 1985. The preferential translation of Drosophila hsp70 mRNA requires sequences in the untranslated leader. *Cell* 42:903–11

162. McKay DB. 1991. Structure of the 70-kilodalton heat-shock-related proteins. *Semin. Immunopathol.* 13:1–9

163. McLaughlin MM, Bossard MJ, Koser PL, Cafferkey R, Morris RA, et al. 1992. The yeast cyclophilin multigene family: purification, cloning and characterization of a new isoform. *Gene* 111:85–92

164. McMullin TW, Hallberg RL. 1987. A normal mitochondrial protein is selectively synthesized and accumulated during heat shock in *Terrahymena thermophila. Mol. Cell Biol.* 7:4414–23

165. McMullin TW, Hallberg RL. 1988. A highly evolutionarily conserved mitochondrial protein is structurally related to the protein encoded by the *Escherichia coli groEL* gene. *Mol. Cell Biol.* 8:371–80

166. Mendelsohn ME, Zhu Y, ONeill S. 1991. The 29-kDa proteins phosphorylated in thrombin-activated human platelets are forms of the estrogen receptor-related 27-kDa heat shock protein. *Proc. Natl. Acad. Sci. USA* 88:11212–16

167. Mendoza JA, Rogers E, Lorimer GH, Horowitz PM. 1991. Chaperonins facilitate the in vitro folding of monomeric mitochondrial rhodanese. *J. Biol. Chem* 266:13044–49

168. Mendoza JA, Rogers E, Lorimer GH, Horowitz PM. 1991. Unassisted refolding of urea unfolded rhodanese. *J. Biol. Chem.* 266:13587–91

169. Merck KB, Groenen PJ, Voorter CE, de Haard Hoekman WA, Horwitz J, et al. 1993. Structural and functional similarities of bovine alpha-crystallin and mouse small heat-shock protein. A family of chaperones. *J. Biol. Chem* 268:1046–52

170. Miklos D, Caplan S, Mertens D, Hynes V, Pitluk Z, et al. 1993. A second member of the TCP1 complex of yeast, TCP1/3. *Proc. Natl. Acad. Sci. USA* 268: In press

171. Meyers G, Rumenapf T, Theil H. 1989. Ubiquitin in a togavirus. *Nature* 341:491

172. Minami Y, Kawasaki H, Suzuki K, Yahara I. 1993. The calmodulin-binding domain of the mouse 90-kDa heat shock protein. *J. Biol. Chem.* 268:9604–10

173. Miron T, Vancompernolle K, Vandekerckhove J, Wilchek M, Geiger B. 1991. A 25-kD inhibitor of actin polymerization is a low molecular mass heat shock protein. *J. Cell Biol.* 114:255–61

174. Miyata Y, Yahara I. 1991. Cytoplasmic 8 S glucocorticoid receptor binds to actin filaments through the 90-kDa heat shock protein moiety. *J. Biol. Chem.* 266:8779–83

175. Miyata Y, Yahara I. 1992. The 90-kDa heat shock protein, HSP90, binds and protects casein kinase II from self-aggregation and enhances its kinase activity. *J. Biol. Chem.* 267:7042–47

176. Mori K, Sant A, Kohno K, Normington K, Gething M-J, et al. 1992. A 22 bp cis-acting element is necessary and sufficient for the induction of the yeast *KAR2* (BiP) gene by unfolded proteins. *EMBO J.* 11:2583–93

177. Muller WU, Li GC, Goldstein LS. 1985. Heat does not induce synthesis of heat shock proteins or thermotolerance in the earliest stage of mouse embryo development. *Int. J. Hyperthermia* 1:97–102

177a. Mummert E, Grimm R, Speth V, Eckerskorn C, Schlitz E, et al. 1993. A TCP-1related molecular chaperone from plants refolds phytochrome to its photoreversible form. *Nature* 363:644–48

178. Munro S, Pelham HR. 1986. An Hsp70-like protein in the ER: identity with the 78 kd glucose-regulated protein and immunoglobulin heavy chain binding protein. *Cell* 46:291–300

179. Nadeau K, Das A, Walsh CT. 1993. Hsp90 chaperonins possess ATPase activity and bind heat shock transcription factors and peptidyl prolyl isomerases. *J. Biol. Chem* 268:1479–87

180. Nagao RT, Kimpel JA, Vierling E, Key JL. 1986. The Heat Shock Response: A Comparative Analysis. In *Oxford Surveys of Plant Molecular & Cell Biology*, pp. 384–438. Oxford Univ. Press

181. Nakai A, Satoh M, Hirayoshi K, Nagata K. 1992. Involvement of the stress protein hsp47 in procollagen processing in the endoplasmic reticulum. *J. Cell Biol.* 117:903–14

182. Neidhardt FC, VanBogelen RA, Vaughn V. 1984. The genetics and regulation of heat-shock proteins. *Annu. Rev. Genet.* 18:295–329

183. Nelson RJ, Ziegelhoffer T, Nocolet C, Werner-Washburne M, Craig EA. 1992. The translation machinery and 70 kd heat shock protein cooperate in protein synthesis. *Cell* 71:97–105

184. Neupert W, Hartl FU, Craig EA, Pfanner N. 1990. How do polypeptides cross the mitochondrial membranes? *Cell* 63:447–50

185. Nilsson B, Anderson S. 1991. Proper and improper folding of proteins in the cellular environment. *Annu. Rev. Microbiol.* 45:607–35

186. Ning Y-M, Sanchez ER. 1993. Potentiation of glucocorticoid receptor-mediated gene expression by the immunophilin ligands FK506 and rapamycin. *J. Biol. Chem.* 268:6073–76

187. Nishida E, Koyasu S, Sakai H, Yahara I. 1986. Calmodulin-regulated binding of the 90-kDa heat shock protein to actin filaments. *J. Biol. Chem* 261:16033–36

188. Nover L, ed. 1991. *Heat Shock Response*. Boca Raton, FL: CRC Press. 1st ed.

189. OBrien T, Lis JT. 1991. RNA polymerase II pauses at the 5' end of the transcriptionally induced Drosophila hsp70 gene. *Mol. Cell Biol.* 11:5285–90

189a. Ou W-J, Cameron PH, Thomas DY, Bergeron JJM. 1993. Association of folding intermediates of glycoproteins with calnexin during protein maturation. *Nature* 364:771–76

190. Ozkaynak E, Finley D, Varshavsky A. 1984. The yeast ubiquitin gene: head-to-tail repeats encoding a polyubiquitin precursor protein. *Nature* 312:663–66

191. Paliwal BR, Hetzel F, Dewhirst MW, eds. 1988. *Biological, Physical and Clinical Aspects of Hyperthermia*. AAPM Monogr. Vol. 16. New York: Am. Inst. Physics

192. Palleros DR, Welch WJ, Fink AL. 1991. Interaction of hsp70 with unfolded proteins: effects of temperature and nucleotides on the kinetics of binding. *Proc. Natl. Acad. Sci. USA* 88:5719–23

192a. Palleros DR, Reid KL, Shi L, Welch WJ, Fink AL. 1993. ATP-induced protein-hsp70 complex dissociation requires K+ and does not involve ATP hydrolysis. Analogy to G proteins. *Nature* In press

193. Parag HA, Raboy B, Kulka RG. 1987. Effect of heat shock on protein degradation in mammalian cells: involvement of the ubiquitin system. *EMBO J.* 6:55–61

194. Parker CS, Topol J. 1984. A Drosophila RNA polymerase II transcription factor binds to the regulatory site of an hsp 70 gene. *Cell* 37:273–83

195. Parsell DA, Sanchez Y, Stitzel JD, Lindquist S. 1991. Hsp104 is a highly conserved protein with two essential nucleotide-binding sites. *Nature* 353:270–73

196. Parsell DA, Sauer RT. 1989. Induction of a heat shock-like response by unfolded protein in *Escherichia coli*: dependence on protein level not protein degradation. *Genes Dev.* 3:1226–32

197. Parsell DA, Taulien J, Lindquist S. 1993. The role of heat-shock proteins in thermotolerance. *Philos. Trans. R. Soc. London Ser. B* 339:279–86

197a. Pelham HR. 1984. Hsp70 accelerates the recovery of nucleolar morphology after heat shock. *EMBO J* 3:3095–100

198. Pelham HR. 1986. Speculations on the functions of the major heat shock and glucose-regulated proteins. *Cell* 46:959–61

198a. Pelham HR, Lewis MJ, Lindquist S. 1984. Expression of a Drosophila heat shock protein in mammalian cells: transient association with nucleoli after heat shock. *Philos. Trans. R. Soc. London (Biol.)* 307:301–7

199. Petersen R, Lindquist S. 1988. The Drosophila hsp70 message is rapidly degraded at normal temperatures and stabilized by heat shock. *Gene* 72:161–68

200. Petko L, Lindquist S. 1986. Hsp26 is not required for growth at high temperatures, nor for thermotolerance, spore development, or germination. *Cell* 45:885–94

201. Phillips TA, VanBogelen RA, Neidhardt FC. 1984. lon gene product of *Escherichia coli* is a heat-shock protein. *J. Bacteriol.* 159:283–87

202. Phipps BM, Typke D, Heger R, Volker S, Hoffmann A, et al. 1993. Structure of a molecular chaperone from a thermophilic archaebacterium. *Nature* 361:475–77

203. Picard D, Khursheed B, Garabedian MJ, Fortin MG, Lindquist S, et al. 1990. Reduced levels of hsp90 compromise steroid receptor action in vivo. *Nature* 348:166–68

204. Pine MJ. 1967. Response of intracellular proteolysis to alteration of bacterial protein and the implications in metabolic regulation. *J. Bacteriol.* 93:1527–33

205. Plesset J, Ludwig JR, Cox BS, McLaughlin CS. 1987. Effect of cell cycle position on thermotolerance in *Saccharomyces cerevisiae*. *J. Bacteriol.* 169:779–84

206. Pratt WB, Hutchison KA, Scherrer LC. 1992. Steroid receptor folding by heat-shock proteins and composition of the receptor heterocomplex. *Trends Exp. Med.* 3:326–33

207. Pratt WB, Scherrer LC, Hutchison KA, Dalman FC. 1992. A model of glucocorticoid receptor unfolding and stabilization by a heat shock protein complex. *J. Steroid Biochem. Mol. Biol.* 41:223–29

208. Reading DS, Hallberg RL, Myers AM. 1989. Characterization of the yeast HSP60 gene coding for a mitochondrial assembly factor. *Nature* 337:655–59

209. Rechsteiner M, Hoffman L, Dubiel W. 1993. The multicatalytic and 26S proteases. *J. Biol. Chem.* 265:6065–68

210. Redmond T, Sanchez ER, Bresnick EH, Schlesinger MJ, Toft DO, et al. 1989. Immunofluorescence colocaliza-

tion of the 90-kDa heat-shock protein and microtubules in interphase and mitotic mammalian cells. *Eur. J. Cell Biol.* 50:66–75

211. Resendez EJ, Wooden SK, Lee AS. 1988. Identification of highly conserved regulatory domains and protein-binding sites in the promoters of the rat and human genes encoding the stress-inducible 78-kilodalton glucose-regulated protein. *Mol. Cell. Biol.* 8:4579–84

212. Riabowol KT, Mizzen LA, Welch WJ. 1988. Heat shock is lethal to fibroblasts microinjected with antibodies against hsp70. *Science* 242:433–36

212a. Rippmann F, Taylor WR, Rothbard JB, Green NM. 1991. A hypothetical model for the peptide binding domain of hsp70 based on the peptide binding domain of HLA. *EMBO J* 10:1053–59

213. Rose DW, Welch WJ, Kramer G, Hardesty B. 1989. Possible involvement of the 90-kDa heat shock protein in the regulation of protein synthesis. *J. Biol. Chem* 264:6239–44

214. Rossi JM, Lindquist S. 1989. The intracellular location of yeast heat-shock protein 26 varies with metabolism. *J. Cell Biol.* 108:425–39

215. Rothman JE. 1989. Polypeptide chain binding proteins: catalysts of protein folding and related processes in cells. *Cell* 59:591–601

216. Rothman JE, Schmid SL. 1986. Enzymatic recycling of clathrin from coated vesicles. *Cell* 46:5–9

217. Saibil HR, Zheng D, Roseman AM, Hunter AS, Watson GME, et al. 1993. ATP induces large quaternary rearrangements in a cage-like chaperonin structure. *Curr. Biol.* 3:265–73

218. Sanchez Y, Lindquist S. 1990. HSP104 required for induced thermotolerance. *Science* 248:1112–15

219. Sanchez Y, Parsell DA, Taulien J, Vogel JL, Craig EA, et al. 1993. Genetic evidence for a functional relationship between hsp104 and hsp70. *J. Bacteriol.* In press

220. Sanchez Y, Taulien J, Borkovich KA, Lindquist S, et al. 1992. Hsp104 is required for tolerance to many forms of stress. *EMBO J.* 11:2357–64

221. Sander C, Schneider R. 1991. Database of homology-derived protein structures. *Proteins* 9:56–68

222. Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. 1990. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 63:1129–36

223. Schein CH. 1989. Production of soluble

recombinant proteins in bacteria. *Biotechnology* 7:1141–49

224. Schenberg-Frascino A, Moustacchi E. 1972. Lethal and mutagenic effect of elevated temperature on haploid yeast I. Variations in sensitivity during the cell cycle. *Mol. Gen. Genet.* 115:243–57

225. Scherrer LC, Dalman FC, Massa E, Meshinchi S, Pratt WB. 1990. Structural and functional reconstitution of the glucocorticoid receptor-hsp90 complex. *J. Biol. Chem.* 265:21397–400

226. Schmid FX, Mayr L, Mucke M, Schonbrunner ER. 1992. Prolyl isomerases: the role in protein folding. *Adv. Protein Chem.* In press

227. Schreiber SL, Crabtree GR. 1992. The mechanism of action of cyclosporin A and FK506. *Immunol. Today* 13:136–42

228. Schroder H, Langer T, Hartl FU, Bukau B. 1993. DnaK, DnaJ, GrpE form a cellular chaperone machinery capable of repairing heat-induced protein damage. *EMBO J.* 12: In press

229. Schwartz AL, Ciechanover A, Brandt RA, Geuze HJ. 1988. Immunoelectron microscopic localization of ubiquitin in hepatoma cells. *EMBO J.* 7:2961–66

230. Sconzo G, Roccheri MC, La RM, Oliva D, Abrignani A, et al. 1986. Acquisition of thermotolerance in sea urchin embryos correlates with the synthesis and age of the heat shock proteins. *Cell Differ.* 19:173–77

231. Seaton BL, Vickery LE. 1993. A gene encoding a new DnaK/hsp70 homolog in *Escherichia coli. Proc. Natl. Acad. Sci. USA.* Manuscript submitted.

232. Seufert W, Jentsch S. 1990. Ubiquitin-conjugating enzymes UBC4 and UBC5 mediate selective degradation of short-lived and abnormal proteins. *EMBO J.* 9:543–50

233. Shanklin J, Jabben M, Vierstra RD. 1987. Red light-induced formation of ubiquitin-phytochrome conjugates: identification of possible intermediates of phytochrome degradation. *Proc. Natl. Acad. Sci. USA* 84:359–63

234. Sherman M, Goldberg AL. 1992. Heat shock in *Escherichia coli* alters the protein-binding properties of the chaperonin groEL by inducing its phosphorylation. *Nature* 357:167–69

234a. Sherman M, Goldberg AL. 1992. Involvement of the chaperonin dnaK in the rapid degradation of a mutant protein in *Escherichia coli. EMBO J.* 11:71–77

235. Sherman MY, Goldberg AL. 1991. Formation in vitro of complexes be-

tween an abnormal fusion protein and the heat shock proteins from *Escherichia coli* and yeast mitochondria. *J. Bacteriol.* 173:7249–56

236. Sigal NH, Dumont F, Durette P, Sieierka JJ, Peterson L, et al. 1991. Is cyclophilin involved in the immunosuppressive and nephrotoxic mechanism of action of cyclosporin A? *J. Exp. Med.* 173:619–28

237. Skowyra D, Georgopoulos C, Zylicz M. 1990. The *E. coli dnaK* gene product, the hsp70 homolog, can reactivate heat-inactivated RNA polymerase in an ATP hydrolysis-dependent manner. *Cell* 62:939–44

238. Smith DF, Stensgard BA, Welch WJ, Toft DO. 1992. Assembly of progesterone receptor with heat shock proteins and receptor activation are ATP mediated events. *J. Biol. Chem* 267:1350–56

239. Smith DF, Toft DO. 1993. Steroid receptors and their associated proteins. *Mol. Endocrinol.* 7:4–11

240. Solomon JM, Rossi JM, Golic K, McGarry T, Lindquist S. 1991. Changes in Hsp70 alter thermotolerance and heat-shock regulation in Drosophila. *New Biol.* 3:1106–20

241. Squires C, Squires CL. 1992. The Clp proteins: Proteolysis regulators or molecular chaperones? *J. Bacteriol.* 174:1081–85

242. Squires CL, Pedersen S, Ross BM, Squires C. 1991. ClpB is the *Escherichia coli* heat shock protein F84.1. *J. Bacteriol.* 173:4254–62

243. Stamnes MA, Shieh BH, Chuman L, Harris GL, Zuker CS. 1991. The cyclophilin homolog ninaA is a tissue-specific integral membrane protein required for the proper synthesis of a subset of Drosophila rhodopsins. *Cell* 65:219–27

244. Steinmann B, Bruckner P, Supertii-Furga A. 1991. Cyclosporin A slows collagen triple-helix formation in vivo: Indirect evidence for a physiologic role of peptidyl-prolyl cis-trans-isomerase. *J. Biol. Chem.* 266:1299–303

245. Stewart DE, Sarkar A, Wampler JE. 1990. Occurrence and role of cis peptide bonds in protein structures. *J. Mol. Biol.* 214:253–60

246. Strauch KL, Beckwith J. 1988. An *Escherichia coli* mutation preventing degradation of abnormal periplasmic proteins. *Proc. Natl. Acad. Sci. USA* 85:1576–80

247. Strauch KL, Johnson K, Beckwith J. 1989. Characterization of *degP*, a gene required for proteolysis in the cell

envelope and essential for growth of *Escherichia coli* at high temperature. *J. Bacteriol.* 171:2689–96

248. Straus D, Walter WA, Gross CA. 1990. DnaK, DnaJ, GrpE heat shock proteins negatively regulate heat shock gene expression by controlling the synthesis and stability of sigma 32. *Genes Dev.* 4:2202–9

249. Straus DB, Walter WA, Gross CA. 1988. *Escherichia coli* heat shock gene mutants are defective in proteolysis. *Genes Dev.* 2:1851–8

250. Subjeck JR, Shyy TT. 1986. Stress protein systems of mammalian cells. *Am. J. Physiol.* 250:C1–17

251. Susek RE, Lindquist SL. 1989. hsp26 of *Saccharomyces cerevisiae* is related to the superfamily of small heat shock proteins but is without a demonstrable function. *Mol. Cell Biol.* 9:5265–71

252. Sykes K, Gething M-J, Sambrook J. 1993. Proline isomerases function during heat shock. *Proc. Natl. Acad. Sci. USA* 90:5853–57

253. Deleted in proof

254. Tai PK, Albers MW, Chang H, Faber LE, Schreiber SL. 1992. Association of a 59-kilodalton immunophilin with the glucocorticoid receptor complex. *Science* 256:1315–18

255. Tai PK, Chang H, Albers MW, Schreiber SL, Toft DO, et al. 1993. P59 (FKBP59) heat shock protein interaction is highly conserved and may involve proteins other than steroid receptors. *Biochemistry* In press

256. Tierney DJ, Haas AL, Koop DR. 1992. Degradation of cytochrome P450 2E1: selective loss after labilization of the enzyme. *Arch. Biochem. Biophys.* 293:9–16

257. Tilly K, McKittrick N, Zylicz M, Georgopoulos C. 1983. The dnaK protein modulates the heat-shock response of *Escherichia coli. Cell* 34:641–6

258. Tobias JW, Shrader TE, Rocap G, Varshavsky A. 1991. The N-end rule in bacteria. *Science* 254:1374–77

258a. Toledo H, Carlino A, Vidal V, Redfield B, Nettleton MY, Kachan JP, et al. 1993. Dissociation of glucose-regulated protein Grp78 and Grp78-IgE Fc Complexes by ATP. *Proc. Nat. Assoc. Sci. USA* 90:2505–8

259. Toniolo D, Persico M, Alcalay M. 1988. A "housekeeping" gene on the X chromosome encodes a protein similar to ubiquitin. *Proc. Natl. Acad. Sci. USA* 85:851–55

260. Trent JD, Nimmesgern E, Wall JS, Hartl FU, Horwich AL. 1991. A molecular chaperone from a thermophilic

archaebacterium is related to the eu-
karyotic protein t-complex polypeptide-
1. *Nature* 354:490–93

261. Trent JD, Osipiuk J, Pinkau T. 1990.
Acquired thermotolerance and heat
shock in the extremely thermophilic
archaebacterium *Sulfolobus sp.* strain
B12. *J. Bacteriol.* 172:1478–84

262. Tuite MF, Bentley NJ, Bossier P, Fitch
IT. 1990. The structure and function
of small heat shock proteins: analysis
of the *Saccharomyces cerevisiae* Hsp26
protein. *Antonie Van Leeuwenhoek* 58:
147–54

263. Ursic D, Culbertson MR. 1991. The
yeast homolog to mouse Tcp-1 affects
microtubule-mediated processes. *Mol.
Cell Biol.* 11:2629–40

263a. Ursic D, Culbertson MR. 1992. Is
yeast TCP1 a chaperonin? *Nature* 356:
392

264. Van Dyk TK, Gatenby AA, LaRossa
RA. 1989. Demonstration by genetic
suppression of interaction of GroE
products with many proteins. *Nature*
342:451–53

264a. Velazquez JM, Lindquist S. 1984.
Hsp70: nuclear concentration during
environmental stress and cytoplasmic
storage during recovery. *Cell* 36:655–
62

265. Velazquez JM, Sonoda S, Bugaisky
G, Lindquist S. 1983. Is the major
Drosophila heat shock protein present
in cells that have not been heat
shocked? *J. Cell Biol.* 96:286–90

266. Vierling E. 1991. The roles of heat
shock proteins in plants. *Annu. Rev.
Plant Physiol. Plant Mol. Biol.* 42:579–
620

267. Viitanen PV, Donaldson GK, Lorimer
GH, Lubben TH, Gatenby AA. 1991.
Complex interactions between the chap-
eronin 60 molecular chaperone and
dihydrofolate reductase. *Biochemistry*
30:9716–23

267a. Viitanen PV, Lorimer GH, Seetharam
R, Gupta RS, Oppenheim J, et al.
1992. Mammalian mitochondrial chap-
eronin 60 functions as a single toroidal
ring. *J. Biol. Chem.* 267:695–8

268. Viitanen PV, Lubben TH, Reed J,
Goloubinoff P, O'Keefe DP, et al.
1990. Chaperonin-facilitated refolding
of ribulosebisphosphate carboxylase
and ATP hydrolysis by chaperonin 60
(groEL) are K+ dependent. *Biochem-
istry* 29:5665–71

268a. Vogel JP, Misra LM, Rose MD. 1990.
Loss of BiP/GRP78 function blocks
translocation of secretory proteins in
yeast. *J. Cell Biol.* 110:1885–95

269. Walsh D, Li K, Crowther C, Marsh
D, Edwards M. 1991. Thermotolerance
and heat shock response during early
development of the mammalian em-
bryo. *Results Probl. Cell Differ.* 17:58–
70

270. Waxman L, Goldberg AL. 1982. Pro-
tease La from *Escherichia coli* hydro-
lyzes ATP and proteins in a linked
fashion. *Proc. Natl. Acad. Sci. USA*
79:4883–87

271. Waxman L, Goldberg AL. 1985. Pro-
tease La, the *lon* gene product, cleaves
specific fluorogenic peptides in an
ATP-dependent reaction. *J. Biol.
Chem.* 260:12022–8

272. Weijland A, Parmeggiani A. 1993.
Toward a model for the interaction
between elongation factor Tu and the
ribosome. *Science* 259:1311–14

273. Welch WJ. 1991. The role of heat-
shock proteins as molecular chaper-
ones. *Curr. Opin. Cell Biol.* 3:1033–
38

273a. Welch WJ, Feramisco JR. 1984. Nu-
clear and nucleolar localization of the
72,000-dalton heat shock protein in
heat-shocked mammalian cells. *J. Biol.
Chem.* 259:4501–13

274. Welte MA, Tetrault JM, Dellavalle
RP, Lindquist SL. 1993. A new method
for manipulating transgenes: Improving
thermotolerance in Drosophila em-
bryos. *Genes Dev.* Manuscript submit-
ted

275. Deleted in proof

276. Werner-Washburne M, Stone DE,
Craig EA. 1987. Complex interactions
among members of an essential sub-
family of hsp70 genes in *Saccharomy-
ces cerevisiae. Mol. Cell Biol.* 7:
2568–77

277. Wickner S, Hoskins J, McKenney K.
1991. Function of DnaJ and DnaK as
chaperones in origin-specific DNA
binding by RepA. *Nature* 350:165–67

278. Wickner S, Hoskins J, McKenney K.
1991. Monomerization of RepA dimers
by heat shock proteins activates binding
to DNA replication origin. *Proc. Natl.
Acad. Sci. USA* 88:7903–7

279. Wiech H, Buchner J, Zimmermann R,
Jakob U. 1992. Hsp90 chaperones pro-
tein folding in vitro. *Nature* 358:169–
70

280. Woo KM, Kim KI, Goldberg AL, Ha
DB, Chung CH. 1992. The heat-shock
protein ClpB in *Escherichia coli* is a
protein-activated ATPase. *J. Biol.
Chem.* 267:20429–34

281. Xu Y, Lindquist S. 1993. HSP90
governs the activity of p60v-src kinase.
Proc. Natl. Acad. Sci. USA. 90:7074–
78

282. Yaffe MB, Farr GW, Miklos D, Horwich AL, Sternlicht ML, et al. 1992. TCP1 complex is a molecular chaperone in tubulin biogenesis (see comments). *Nature* 358:245–48

283. Yahara I, Iida H, Koyasu S. 1986. A heat shock-resistant variant of Chinese hamster cell line constitutively expressing heat shock protein of Mr 90,000 at high level. *Cell Struct. Funct.* 11:65–73

284. Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN. 1982. Living with water stress: evolution of osmolyte systems. *Science* 217:1214–22

285. Yost HJ, Lindquist S. 1986. RNA splicing is interrupted by heat shock and is rescued by heat shock protein synthesis. *Cell* 45:185–93

286. Yu M-H, King J. 1988. Surface amino acids as sites of temperature-sensitive folding mutations in the P22 tailspike protein. *J. Biol. Chem.* 263:1424–31

287. Zambrano MM, Siegele DA, Almiron M, Tormo A, Kolter R. 1993. Microbial competition: Escherichia coli mutants that take over stationary phase cultures. *Science* 259:1757–60

288. Zimarino V, Wu C. 1987. Induction of sequence-specific binding of Drosophila heat shock activator protein without protein synthesis. *Nature* 327:727–30

289. Zimmerman JL, Petri W, Meselson M. 1983. Accumulation of a specific subset of *D. melanogaster* heat shock mRNAs in normal development without heat shock. *Cell* 32:1161–70

290. Zydowsky LD, Etzkorn FA, Chang MY, Ferguson SB, Stotz JA, et al. 1992. Active site mutants of human cyclophilin A separate PPI activity from CsA binding and calcineurin inhibition. *Protein Sci.* 1:1092–99

291. Zylicz M. 1993. The *Escherichia coli* chaperones involved in DNA replication. *Philos. Trans. R. Soc. London Ser. B.* 339:271–76